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ESTIMATION OF CRITICAL TORQUE USING NEUROMUSCULAR ELECTRICAL STIMULATION OF THE QUADRICEPS IN HUMANS

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ESTIMATION OF CRITICAL TORQUE USING NEUROMUSCULAR ELECTRICAL STIMULATION OF THE QUADRICEPS IN HUMANS

A THESIS APPROVED FOR THE
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ABSTRACT

Critical torque (CT) is an integrative measure/concept that represents the "critical" or upper boundary of steady-state work that can be performed without leading to exhaustive fatigue. While this concept has been observed across multiple voluntary exercise modalities, it has not been tested using neuromuscular electrical stimulation (NMES). **PURPOSE**: The purposes of this study were to 1) observe if an electrically stimulated exercise protocol in the quadriceps results in a hyperbolic power-duration pattern seen in voluntary contractions, and 2) determine if the decline in torque production over time during electrically stimulated exercise occurred due to similar mechanism(s) as the decline in torque during voluntary exercise. **METHODS**: Participants (Men = 6, Women = 8) completed 2 familiarizations and 3 testing visits. Voluntary CT (VOL) and involuntary end-test torque (ETT) were assessed at several frequencies including 100 Hz, an intermediate frequency (Intermediate; 15-30 Hz) and a frequency that elicited a torque below the ETT of 100 Hz (Below; <15 Hz). Twitch torque (TT), low frequency fatigue (LFF), M-wave amplitude, and lactate were measured during each exercise protocol, and %ACT was assessed during the VOL test. **RESULTS**: ETT was calculated as the mean peak of the last 7 contractions for the stimulated exercises. Mean and relative ETT was significantly different from starting torque for each of the 100 Hz and Intermediate (15-30 Hz) protocols ($p \leq 0.002$), but ETT was not significantly different across protocols ($p \geq 0.127$). ETT of the Below protocol did not change from starting ($p \geq 0.558$), and ETT was significantly lower than any of the other stimulated protocols ($p \leq 0.035$).VOL TT declined approximately 60% from starting TT ($p \leq 0.014$). TT declined about 50-60% during the 100 Hz protocols (p

≤ 0.018). Except for transient increases in the middle of the protocol, there were no changes in TT for the Below protocol ($p \geq 0.052$).VOL LFF ratio was significantly reduced from pre exercise measures at all post exercise time points ($p \leq 0.004$). There was acute high frequency fatigue immediately after all of the stimulated protocols ($p \leq 0.040$). M-wave amplitude decreased about 10% from pre to immediately post (IP) for the 100 Hz protocols ($p \leq 0.027$). There were no changes in M-wave amplitude for VOL, Intermediate, and Below protocols ($p \geq 0.19$). Lactate levels during VOL were significantly higher than of all of the stimulated protocols IP and 3-min post (3P) exercise ($p \leq 0.001$). The 100 Hz protocols were significantly higher than the Below protocol at both IP and 3P measures ($p \leq 0.018$). The 100 Hz protocols were not significantly different from the Intermediate protocol at IP ($p \geq 0.59$), but D2 was significantly higher than Intermediate at 3P ($p = 0.044$), while D1 was not ($p = 0.598$). Lactate during IF was not significantly higher than Below at IP ($p = 0.234$), but was higher at 3P ($p = 0.017$). **CONCLUSIONS**: Despite differences in fatigue mechanisms, NMES exercise at 100 Hz and at intermediate frequencies declined to a similar torque value, while exercise under ETT showed no declines in torque. These findings suggest that the observed threshold during NMES exercise is CT.

Chapter 1 – Introduction

1.1 Introduction

Exercise performance, specifically endurance exercise performance, is determined by a host of physiological (e.g. muscle fiber type, mitochondrial number and density, cardiac output, VO_2 peak) and psychological variables (e.g motivation, RPE, muscle pain). Fatigue, defined as a failure to produce the desired or expected force, is a major factor that limits exercise performance. Fatigue that occurs proximal to the neuromuscular junction can be referred to as central fatigue, which involves decline in voluntary motor-unit recruitment and/or activation of skeletal muscle due to decreased signaling from the motor cortex, the cervicomedullary region of the brain, and/or the spinal cord [1]. Fatigue of this type can be influenced by a host of factors including feedback from afferent nerves monitoring the cellular and biochemical environment of the exercising muscles [2]. Conversely, peripheral fatigue occurs distal to the neuromuscular junction and can include failures of acetylcholine release, impairments in calcium release, excitation contraction coupling, and impairments of cross-bridge formation and function [1, 3].

Critical power (CP) is an integrative measure/concept that represents the "critical" or upper boundary of steady-state work that can be performed without leading to fatigue or task failure [4]. CP has been demonstrated to be a better predictor of endurance exercise performance than VO_2 peak and work-rate at lactate threshold [5]. Research has demonstrated that exercise above critical power leads to fatigue in a predictable manner—often termed the power-duration relationship where the higher the work-rate is above CP, the shorter the exercise duration. Exercise at or below CP allows for the attainment of a metabolic (denoted by VO_2 and blood lactate) steady state

in which fatigue does not limit exercise duration [6]. The power-duration relationship and exercise time to exhaustion (T_{lim}) can be modeled using the following equation [6]:

$$T_{lim} = W' / (P - CP)$$

Where W' , the curvature constant above CP, encompasses the total work, measured in Joules, which can be performed above CP, and is a function of exercise duration and power output (P). T_{lim} can be impacted by either the magnitude of P or W' . For example, a higher power output would result in a more rapid time-to-exhaustion, as W' is depleted more rapidly. Conversely, a larger W' would allow for greater work to be performed above CP prior to exhaustion.

While the physiological and psychological determinants of CP are not fully understood, recent research has expanded the understanding of the role of peripheral fatigue on critical power. Voluntary exercise at CP has been shown to reach a steady state in VO_2, blood lactate, and pulmonary ventilation. However, exercise above CP results in progressive increases in blood lactate and progressive increases in VO_2 such that VO_2 peak will be reached [7]. Additionally, fatigue above critical power has also been shown to be associated with the depletion of PCr, the build-up of inorganic phosphate (P_i), and a decrease in pH in the muscle [8].

Several recent studies by Amann [2, 9, 10] have demonstrated the important role of feedback from group III and IV afferent nerve fibers in muscular fatigue and exercise time-to-exhaustion. Group III and IV afferent fibers "sense" the build-up of certain metabolic by-products, which can then lead to the development of "central" fatigue via a reduction in central motor drive from the cortex and spinal motoneurons [10-13].

Thus, the accumulation of peripheral metabolites can reduce exercise performance via a central, rather than a peripheral mechanism. Since critical power plays an important role in determining exercise performance, it is plausible group III and IV afferent signaling may play a role in determining CP. However, it is difficult to delineate the independent effects of central fatigue and peripheral fatigue during voluntary exercise.

Traditionally, CP has been assessed by performing multiple exercise bouts at various intensities, presumably over CP, until exhaustion. Each individual test is then plotted to construct a power-duration relationship, from which CP can be derived. Recently, single session, all-out tests have been developed and validated for determining CP across a host of exercise modalities (e.g. running, cycling, and isometric exercise) [14-17]. Burnley [14] has demonstrated a leveling off of torque production during the final minute of a 5-min test where repeated maximal isometric efforts (MVC's) were performed. This end-test torque value was found to be similar to the critical torque (CT) determined using the traditional multiple tests model. Interestingly, several studies [18-20] using electrical stimulated isometric exercise have demonstrated a similar pattern of decline in isometric torque (an initial decline that eventually stabilizes) that appears to mimic the torque-duration relationship observed in the voluntary test of Burnley [14]. However, not enough is yet understood about the similarities in the patterns to suggest that what is observed during electrically stimulated exercise represents CP/CT. If, through further study, it could be determined that the end-test torque observed during electrically stimulated exercise does represent a critical threshold, it could provide a valuable tool to investigate the central and peripheral contributions to CT.

1.2 Research questions

1. Does intermittent electrically stimulated isometric exercise lead to the attainment of a steady-state end-test torque?

   Sub-Questions

   a. Does electrically stimulated exercise, with a similar initial torque, at 100 Hz result in similar end-test torques on different testing days?
   b. Does manipulating initial torque during stimulated exercise, by altering stimulation frequency (from 100 Hz to 15-30 Hz), lead to the attainment of a similar steady-state end-test torque?
   c. Does manipulating initial torque during stimulated exercise, by altering stimulation frequency, such that it is below end-test torque from 100 Hz not result in a decline in torque (no fatigue)?
   d. Does the decline in torque production over time during electrically stimulated exercise occur due to similar mechanism(s) as the decline in torque during voluntary exercise?

1.3 Hypotheses

H_o: Critical torque

1. There will be a plateau in end-test torque during 100 Hz stimulation, determined by no significant difference among the final 7 contractions.
2. There will be a plateau in end-test torque at an intermediate (15-30 Hz) stimulation frequency stimulation, determined by no significant differences among the last 7 contractions.
3. The end-test torques from 100 Hz and the intermediate frequency will not differ.

4. There will be no change over time in torque during stimulation at a frequency that evokes a torque below end-test torque.

H_o: Critical torque

1. There will be a change (decline) in end-test torque at 100 Hz, determined by significant differences among the final 7 contractions.
2. There will be a change (decline) in end-test torque at the intermediate frequency, determined by significant differences among the final 7 contractions.
3. The end-test torques from 100 Hz and the intermediate frequency will be significantly different from each other.
4. There will be a significant change in force over time during stimulation at a frequency that evokes a torque below end-test torque.

H_o: Fatigue mechanisms

1. Normalized M-wave amplitudes will not change over time (pre-test, during, and post-test) for each exercise protocol (voluntary, 100 Hz, the intermediate frequency, and the low frequency).
2. Normalized M-wave amplitudes will not differ among exercise testing protocols for each measurement point (pre-test, during, and post-test).
3. There will be no significant differences in low frequency fatigue between pre-test and post-test for each exercise protocol (voluntary, 100 Hz, the intermediate frequency, and the low frequency).
4. There will be no significant differences in low frequency fatigue among exercise testing protocols for each measurement point (pre-test and post-test).

5. There will be no significant differences in voluntary activation over time during the voluntary exercise protocol.
6. There will be no significant differences in twitch torque over time (pre-test, during, and post-test) for voluntary, 100 Hz, intermediate, and below CT frequency exercise protocols.
7. There will be no significant differences in twitch torque among exercise testing protocols at each measurement point (pre-test, during, and post-test).

H_a : Fatigue mechanisms

1. There will be significant differences in M-wave amplitudes pre-test, during, and post-test for each exercise protocol (voluntary, 100 Hz, the intermediate frequency, and the low frequency).
2. There will be significant differences in M-wave amplitudes between exercise testing protocols for each measurement point (pre-test, during, and post-test).
3. There will be significant differences in low frequency fatigue between pre-test and post-test for each exercise protocol (voluntary, 100 Hz, the intermediate frequency, and the low frequency).
4. There will be significant differences in low frequency fatigue between exercise testing protocols for each measurement point (pre-test and post-test).
5. There will be significant differences in voluntary activation (ITT) between pre-test, during, and post-test during the voluntary exercise protocol.
6. There will be significant differences in twitch torque pre-test, during, and post-test for voluntary, 100 Hz, and the intermediate frequency exercise protocols.

7. There will be significant differences in twitch torque between exercise testing protocols for each measurement point (pre-test, during, and post-test).

1.4 Significance

A passive stimulation exercise that is capable of producing the power-duration relationship would be significant because it removes the voluntary element from the calculation. It would demonstrate that a critical threshold of force or power output could be an inherent property of muscles themselves, and not entirely the result of central fatigue or changes in neural stimulation. As a result, it would allow us to examine the effect of group III and IV afferent feedback on CT via central fatigue.

1.5 Delimitations

The delimitations of this study include the following:

1. Participants are males and females (18-45 years).
2. Participants will be recruited from the University of Oklahoma and the Norman area.
3. Participants will not be eligible for this study if they have any leg injuries that would prevent them from performing a seated knee extension.
4. Participants will not be eligible for this study if they answered “yes” to any of the questions on the physical activity readiness questionnaire (PAR-Q).
5. Participants will not be eligible for this study if they are pregnant or thinking of becoming pregnant.

1.6 Limitations

The limitations of this study include the following:

1. The results of this study can only be applied to healthy males and females, ages 18 to 45 years.

2. Given the differences in recruitment patterns between voluntary and electrical stimulation, the critical torque values derived from NMES cannot be directly related to the values derived from MVCs.

1.7 Assumptions

The assumptions of this study include the following:

1. Participants will adhere to all instructions and guidelines given by the researchers.
2. Participants will provide truthful answers on all questionnaires.

1.8 Definitions of terms

Given the specificity of our work, it would be helpful to define our primary terms:

1. **Critical power (CP):** an integrative measure that represents the critical boundary of steady-state work that can be performed without leading to exhaustive fatigue [4].
2. **Critical torque (CT):** the critical power determined during isometric contraction.
3. **W'** : represents the curvature constant of the relationship. It is a finite amount of work that can be completed above the critical intensity or power [14].
4. **Neuromuscular electrical stimulation (NMES)**: A method of activating muscle by sending electrical current through electrodes on cutaneous surface. Allows for mechanical contraction without voluntary activation.
5. **Low frequency fatigue (LFF)**: the phenomenon in which torque produced in response to low frequency stimulations decline disproportionately to the torque produced in response to higher frequency stimulations [21].

6. **Voluntary activation/interpolated twitch technique (ITT):** A technique used to assess the percentage of skeletal muscle that an individual can voluntarily activate during a muscle contraction [22].
7. **Peripheral fatigue:** Changes within a muscle that impairs torque or force production. Occurs downstream of the neuromuscular junction.
8. **Central fatigue:** Decrease in voluntary activation caused by inhibition of the nervous system. Occurs upstream of the neuromuscular junction.

Chapter 2 – Review of Literature

We searched the MEDLINE and PubMed electronic databases using the search terms critical power and (exercise tolerance) and (determination), critical power/torque and (estimation), critical power/torque and (exercise intensity), and neuromuscular electrical stimulation/electromyostimulation and (MRI). Only articles in English were used for review.

2.1 Electrical stimulation

It is possible to artificially contract the muscle by using neuromuscular electrical stimulation (NMES), which results in the mechanical contraction, even without voluntary activation. Electrical current is run through electrodes placed on the cutaneous surface of the desired muscle, causing a contraction of the associated muscle fibers.

While NMES allows for artificial activation of muscle fibers, it is important to consider the difference in response patterns of NMES and voluntary contraction – primarily that fatigue is achieved sooner during NMES than it is during voluntary contraction. While early research suggested that reversal of recruitment order during NMES, with large diameter, fast twitch fibers being recruited first, was responsible for the discrepancy in fatigue time, it is now generally believed that NMES-induced contractions are non-selective and motor neuron recruitment occurs in the same order as during voluntary activation [23].

A study by Adams et al [24] indicated that the time constant for decay of a magnetic resonance signal increased more in muscles activated by NMES than those by voluntary activation. The authors postulated that this was due to stimulated activation being synchronous, versus the asynchronous activation of voluntary contraction, a

strategy used to prevent fatigue during exercise [24, 25]. In addition, during repeated voluntary contraction, EMG activity increased, indicating increased motor unit recruitment to offset fatigue [26]. In addition to varied recruitment of motor units, increasing the total number of motor units activated during voluntary contraction can function to maintain muscle contraction despite the fatigue of individual motor units. Finally, during voluntary contraction, motor units can be activated at lower frequencies, which also function to offset fatigue [26]. None of these activation strategies appear to be available during NMES. This lack of variability in recruitment of motor units could explain the increased fatigability seen in NMES versus voluntary contraction [25].

As far as we know, no study has tested a method of estimating CP/CT by using NMES. However, a study by Bickel et al. that compared the decline in peak torque for the tibialis anterior muscle and the quadriceps femoris muscle over 180 contractions with NMES show a pattern that strongly resembles the critical torque relationship [18]. Similarly, a study by Russ and Binder-Macleod demonstrated a leveling out of force output during a 180-train fatigue test in the quadriceps [19]. However, it has not been determined if this characteristic of NMES can be described as critical torque.

2.2 Fatigue

Fatigue, in the most basic sense, is the inability to maintain a desired level of intensity or force during exercise. There are many causes and sites of fatigue in skeletal muscle, including within individual muscle cells, the muscle, and extending to the whole body. For a comprehensive review on skeletal muscle fatigue, see Kent-Braun et al [1].

Mechanisms of fatigue are often compartmentalized into two major categories depending on if it occurs proximally (central fatigue) or distally (peripheral fatigue) to the neuromuscular junction.

Peripheral fatigue can be caused by several factors, including impairment of excitation-contraction coupling (altered Ca2+ release from the SR), build-up of metabolites, reduced Ca2+ availability, and depletion of acetylcholine at the neuromuscular junction. While peripheral fatigue appears to occur below CP, it develops at a slow rate that does not lead to task failure [14, 27]. While present, to some extent, during low intensity exercise, peripheral fatigue is often associated with high intensity exercises (>50% of MVC during intermittent contractions)[28].

Central fatigue is associated with decreases in voluntary activation caused by inhibition in the nervous system. Inhibition can occur in the motor cortex, in the cervicomedullary region, and in the spinal cord. The muscular response to electrical stimulation at each of these locations can be measured using surface EMG, indicating the presence and location of central fatigue. Voluntary activation can also be measured using the interpolated twitch technique. Central fatigue is associated with low intensity sustained isometric contractions, approximately 15-30% of MVC [14, 28].

2.3 Assessment of fatigue

M-wave amplitude

Electrical stimulation of the alpha-motoneuron results in a muscle response called the motor wave (M-wave) [29]. A decrease in the amplitude of the M-wave is indicative of neuromuscular transmission failure along the sarcolemma of the muscle cell. Neuromuscular transmission failure can be caused by decreased axonal excitability

(by blockade of axonal propagation of action potentials), by failure of acetylcholine release, by desensitization of acetylcholine receptors on the motor end plate, and reduced excitability of the sarcolemma [30-32]. Presynaptic failure is negligible, and decreases in m-wave amplitude are indicative of transmission failure along the sarcolemma [33].

A study by Takata and Ikata [31] observed greater decreases in M-wave amplitude during high frequency stimulation (100 Hz) than during low frequency stimulation (30 Hz) during 20 minutes of intermittent electrical stimulation of the sciatic nerve in rats. While tension produced during 100 Hz dropped dramatically during the first minutes then leveled out, tension produced by 30 Hz remained relative stable for the duration of the test. These results indicate that neuromuscular transmission failure is a major cause of fatigue during high frequency stimulation.

Traditionally, the M-wave has been elicited by direct stimulation of the peripheral nerve. However, recent research [34] suggests the effectiveness of muscle stimulation to evoke an M-wave that can be used to assess fatigue.

Low frequency fatigue

Low frequency fatigue (LFF) is a phenomenon at which fatigue at low frequencies of stimulation (10 – 40 Hz) increases disproportionately to higher frequencies (>50 Hz) [21]. The effects of LFF can be long-term, lasting up to several days. LFF is generally assessed by comparing the ratios of force or tension produced at a low and high frequency before and after exercise [35].

The causes of LFF are not fully understood and are the topic of much research. Takata and Ikata [31], measured changes in PCr, pH, and M-wave in rat gastrocnemius and soleus muscles during 20 minutes of intermittent electrical nerve stimulation at 100 Hz and 30 Hz. They observed higher levels of PCr depletion and decreases in pH at 30 Hz than at 100 Hz, while m-wave amplitude decreased significantly more for 100 Hz. These more pronounced metabolic changes at lower frequencies led them to postulate that LFF was due, at least in part, to decreases in energy levels and decreases in intracellular pH. While the exact causes of LFF are not yet known, it is likely impairment of excitation-contraction coupling plays a role [36, 37]. Westerblad and colleagues hypothesize that inhibition of Ca2+ release from the sarcoplasmic reticulum is the primary cause of LFF, however, the exact mechanisms of how failure of Ca2+ release induces LFF remain unknown [36, 37].

Voluntary Activation

The interpolated twitch technique (ITT) can be used to calculate voluntary activation and motor unit recruitment using the following formula [22]:

Voluntary activation (%) = 1 – (superimposed doublet/potentiated doublet) x 100

As described earlier, decreases in voluntary activation are indicative of central fatigue. While the ITT can provide valuable information regarding the presence of central fatigue, it does not discern the location, which is an important limitation of this technique.

2.4 Critical power

There are many variables that influence exercise performance and endurance. These factors include physiological factors, such as muscle fiber type, cardiac output,

and VO_2 max, and psychological factors, such as rating of perceived exertion (RPE), motivation, and pain. Critical power is a measure that incorporates these variables to predict exercise performance [4]. Monod and Scherrer [38] were the first to define the CP concept, in which there was a linear relationship between maximum work and the maximal amount of time. They also described the power-duration concept using a hyperbolic relationship, with the curvature of the relationship defined as W' and the asymptote termed CP. They defined critical power as "The maximum rate (of work) that a muscle or muscle group can keep up for a very long time without fatigue [38]." W' is constant and is often associated with the anaerobic work capacity [39]. This, however, is an oversimplification, as evidence suggests that anaerobic work capacity is only part of what determines W' [40]. It is widely believed that the build-ups of metabolites such as inorganic phosphate, H+, and K+ play a role in determining W' [4, 6, 41, 42].

A study by Poole et al [7] examined the physiological responses to exercise at CP compared to those just above CP during exercise on a cycle ergometer. They found that during exercise at CP, blood lactate and VO_2 both reached a steady state and that all participants were able to complete a 24-minute exercise test. However, when exercising just above CP (+5% power), participants' blood lactate increased to intolerance, and VO_2 continuously climbed to VO_{2max}, resulting in termination of the exercise before the 24-minute mark. These findings highlight the nature of CP as a metabolic threshold. Likewise, a study by Jones et al [8] examined the intramuscular metabolic responses to exercise just above and below CP. At exercise below CP, muscle pH, inorganic phosphate, and PCr concentrations reached steady states and participants were able to complete the 20-minute exercise test. At exercise above CP, the same variables

increased until exercise intolerance before the 20-minute mark. These studies provide important insight into some of the factors integrated to determine CP.

Critical power can be manipulated by varying levels of oxygen. A study by Vanhatalo et al [43] indicates that hyperoxia increases CP, while also reducing W’. Muscle PCr and pH dropped at a slower rate, which extended exercise duration, as it took a longer time for these factors to drop to a level associated with intolerance. Reduced oxygen delivery (either hypoxia or blood occlusion) reduces CP [44, 45]. In fact, complete occlusion of the brachial artery had such a reductive effect that CP was measured to be less than zero during handgrip exercises [46], indicating that there is no sustainable metabolic rate under this condition. As would be expected, muscle PCr and pH dropped at a faster rate than during normal, which had a negative effect on exercise tolerance. In these studies, W’ responses to hypoxia varied greatly, ranging from 36% decreases to 66% increases [45]. This fluctuation of W’ as a result of changes in oxygen delivery refutes the idea that W’ is simply a fixed anaerobic work capacity above CP [6].

The critical threshold concept holds when applied to intermittent isometric contractions. When applied to this exercise modality, it is referred to as critical torque (CT). Burnley’s 5-minute all-out test to estimate CT indicated both central and peripheral fatigue during the test. However, the exact locations and mechanisms could not be elucidated [14]. Despite a growing interest in this critical threshold during recent years, much remains unknown regarding determinants of CP/CT and W’.

2.5 Assessment of CP

The criterion method of determining critical intensity has consisted of several – typically four to five – visits where during each visit the participant exercises at a constant intensity at some percentage of max until exhaustion [38, 47]. The time to exhaustion is plotted for every intensity, allowing the power-duration curve to be determined.

Recently, another method of estimating critical intensity has been tested. Vanhatalo et al, found that a three-minute all-out test on a cycle ergometer was a valid and reliable estimator of CP and W' [16, 17]. The concept of a critical intensity is not limited to dynamic activity. Research has been directed toward applying tests of critical intensities to isometric exercise. Burnley applied the all-out test to isometric contraction and found that a five-minute all-out test with a duty cycle of 3 seconds of contraction: 2 seconds of relaxation predicted critical torque in the quadriceps [14]. Conversely, a study by Kellawan and Tschakovsky found that a ten-minute all-out test in the forearm muscles of 10 recreationally active males accurately predicted both critical force and W' [15]. Differences in duty cycles between the two tests could explain some of the mixed results.

Chapter 3 – Methods

3.1 Participants

The sample consisted of 14 volunteers from the University of Oklahoma and Norman area. This sample size was chosen based upon samples used in previous research [14-16] and a power analysis. A sample of 14 was sufficient to detect an effect of ≥ 0.26 SD's using a 4 condition x 3 time point repeated measures ANOVA. Participants provided written informed consent to participate in the study, which was approved by the University of Oklahoma ethics committee and complied with the Declaration of Helsinki. Participants were instructed to refrain from strenuous lower body exercise in the 24 hours preceding each test. Participants were also asked to refrain from caffeine 8 hours before testing and be food-fasted 2 hours before testing.

3.2 Experimental design

A within-participant, repeated measures design, whereby participants served as their own control, was employed. Participants visited the laboratory on five occasions at approximately the same time of day. Familiarization visits occurred at least 24 hours apart, and testing visits occurred at least 48 hours apart.

Visits 1 and 2: Familiarization

Participants were briefed on the study, completed informed consent, and were familiarized with all the equipment and testing procedures during the first and second visits. Participants began by completing a stimulation current determination protocol to determine the amplitude (amount) of electrical current to be used during electrical stimulation (brief 1 ms single pulses of stimulation). Stimulation amplitude was increased until there was a plateau in twitch force, or stimulation was no longer

comfortably tolerable. The participants then performed 3 maximal voluntary isometric contractions (MVC) with their quadriceps. Voluntary activation was determined using the interpolated-twitch technique (ITT) during each MVC. After 10 minutes of rest, participants performed a 3-minute voluntary CT test to familiarize them with the protocol used during the third visit (see "Voluntary all-out test" below). The data from the all-out familiarization test was not be used in analysis. During the second familiarization visit only, the highest tolerable current for a 2-second train of electrical stimulation at 100 Hz was determined for each participant. Additionally, each participant's force-frequency curve was determined using electrical stimulation at his or her highest tolerable level. During the second visit, participants again practiced the voluntary CT test after determination of the force-frequency curve.

Visit 3: Voluntary critical torque assessment

During the third visit, the twitch current was again determined. Participants then completed 3 MVCs with the ITT. Following a 10 minute rest participants then completed a voluntary 5-minute, all-out intermittent test consisting of 60 intermittent MVCs, as described by Burnley [14]. LFF, surface EMG, M-wave, and ITT were recorded at intervals during the voluntary test. Blood lactate, using finger stick, was measured pre-exercise, immediately post-exercise, and 3 minutes post-exercise.

Visits 4 and 5: Electrically stimulated critical torque assessment

During the fourth and fifth visits, the two separate current determination protocols were performed: 1) was completed for use with the ITT for the M-wave assessment (1 ms single pulses) and the LFF assessment (two 1 ms pulses spaced 5 ms

apart); and 2) for a 2-second train at 100 Hz for determining the amplitude of stimulation to be used during the involuntary exercise tests. Participants then completed 3 MVCs with the ITT, after which the train current was determined. Then participants completed a series of 5-minute tests, each of which consisted of a series of 75 intermittent isometric contractions (2 second trains of stimulation followed by 2 seconds of rest) with neuromuscular electrical stimulation (NMES) [see below]. Visit 4 will consist of identical electrical stimulation tests at 100 Hz and a low frequency (Below; <15 Hz), with 30 minutes of rest between each test. LFF, surface EMG, and M-waves were evaluated at intervals during and following exercise. Visit 5 will occur at least 48 hours after visit 4 and consisted of electrical stimulation tests at 100 Hz and an intermediate frequency (15-30 Hz), again, with 30 minutes of rest between each test. LFF, surface EMG and M-wave were recorded during each of the tests. Blood lactate, using finger stick, was measured pre-exercise, immediately post-exercise, and 3 minutes post-exercise for both visits. An overview of the experimental design can be seen in Figure 1.

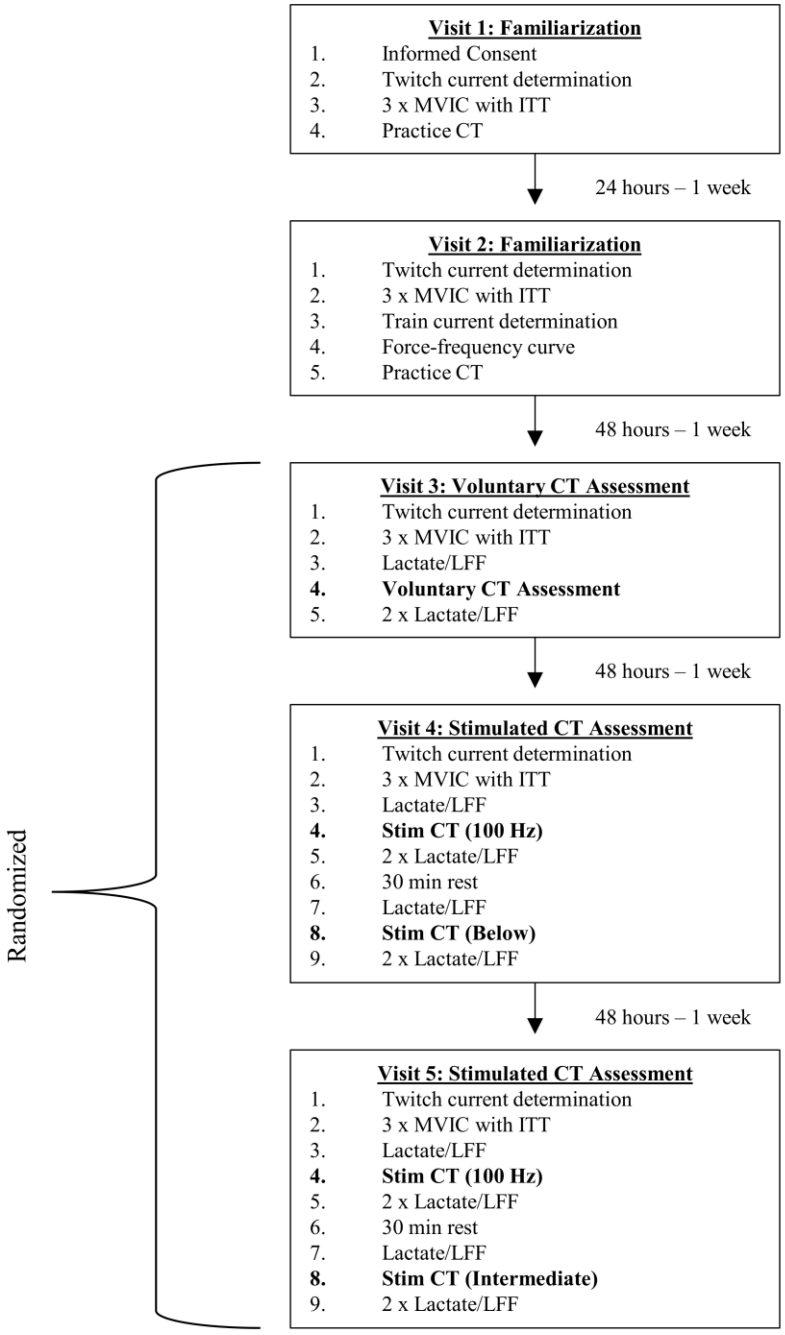


Figure 1 – Overview of experimental procedures.

3.3 Experimental procedures

Dynamometry

All testing was performed using a KinCom isokinetic dynamometer. Participant positioning was similar to previous studies [14, 18]. Participants were seated in the dynamometer and positioned with the hip joint of 85° (with 0° being full extension) and the knee joint angle of 70° below horizontal. The lower leg was strapped to the lever arm at the ankle using a Velcro strap, and the participant was firmly strapped to the seat at the waist and chest. Participant position was marked and recorded to ensure continuity throughout the study.

Maximal voluntary isometric contractions

Participants produced 3 MVC's with their knee extensors. Each contraction lasted ~3 seconds, and 120 seconds of rest was provided between each successive attempt. Participants were cued to contract and relax by verbal instruction from the researcher. Visual biofeedback of their torque was also provided following each attempt. The mean torque value from the plateau region of the force tracing was calculated. Values from 2 efforts that differed by ≤5% were averaged and served as the criterion value for MVC.

Twitch current determination

A current determination protocol was completed at the beginning of each visit to determine the amplitude of stimulation used during the ITT and M-wave measures during MVCs and the voluntary test. Stimulating electrodes were placed over the

proximal vastus lateralis and over the distal vastus medialis. Stimulation electrode positions were marked with indelible ink to ensure continuity throughout the study. Participants first received a single 1 ms twitch/pulse of stimulation at 30 mA. Current amplitude was then progressively increased every 20 seconds by ~20 mA until there was a plateau in the evoked torque production, or until the participant decided that the applied current was no longer comfortable.

Train current determination

At the beginning of the 2nd familiarization and the 4th and 5th testing visits, current for train stimulations was determined. Stimulating electrodes were placed over the proximal vastus lateralis and over the distal vastus medialis. Participants initially received a single, 100 Hz train, for 2-seconds at a current of 30 mA. Stimulation intensity was then progressively increased by 10 mA and additional stimulations will be applied every 20 seconds until the force produced was ~25% of the participant's MVC, or until the participant decided that the applied current would no longer be comfortable when applied for 75 contractions over 5 minutes. The peak current was used for the trains during the force-frequency curve determination and the stimulated/involuntary exercise tests.

Force-frequency curve determination

Constant frequency stimulation trains, using the highest tolerable current, were applied beginning at 5 Hz and increased in increments of 5 Hz until 100 Hz. Each train lasted 2 seconds and there were 30 seconds of rest between each train.

Voluntary all-out test of critical torque

The all-out test was performed in a manner described previously [14]. A 5-minute all-out test consisting of 60 maximal efforts (3 seconds of contraction followed by 2 seconds of rest) was performed. Participants were encouraged to exceed or equal the torque produced by their MVC, but were also informed that their torque will drop across the duration of the test. During the test, participants were verbally encouraged to always attempt a maximal effort. Participants were not informed of the time elapsed or the number of contractions that remained. Participants were cued to contract and relax by a metronome and verbal instruction from the researcher. During this test, ITT was performed during every 6^{th} contraction (every 30 seconds). LFF was evaluated prior to and immediately following the voluntary test. M-wave analysis was performed from the twitches applied during the ITT and the LFF assessments.

Stimulated critical torque assessments

Each participant completed 3 different stimulated tests using constant frequency, constant current trains at 3 different stimulation frequencies. The 3 frequencies used allow for examination of the fatigue characteristics when stimulation occurred at a frequency eliciting peak torque (100 Hz), a frequency eliciting a torque below the end test torque from the 100 Hz protocol (<15 Hz), and a frequency eliciting a torque equal to 50% of the difference between peak torque and end-test torque from the 100 Hz protocol (15-30 Hz). The exercise duration for each test was 5 minutes. Each test consisted of cycles of 2-second trains of stimulation, followed by 2 seconds of rest for a total of 75 contractions. There were 30 minutes of rest between each test. Tests were completed in order of high frequency to low frequency in order to determine each participant's end-test torque for each visit. LFF was evaluated prior to, immediately

after, and 3 minutes after each stimulated test. M-wave analysis was performed prior to, during and following the stimulated exercise by the application of single 1 ms pulses.

Low frequency fatigue

Low frequency fatigue was assessed before, immediately after, and 3 minutes after each voluntary and involuntary test. Each measure was assessed by applying a series of doublets (two 1-millisecond pulses spaced 5 milliseconds apart) and single twitches. Each doublet was paired with a single twitch spaced 3 seconds apart, and each pair was spaced 3 seconds apart. Before the critical torque test, participants received 10 doublet/single stimulation pairs, and the critical torque test was initiated 20 seconds after the final twitch. Two seconds after the conclusion of the critical torque test, 10 more doublet/single pairs were applied. Another 10 pairs were applied 2 minutes later. During the voluntary critical torque visit (visit 3), the stimulation intensity determined during the twitch current determination was used. During the stimulated critical torque visits (visits 4 and 5), the stimulation intensity determined during the train current determination was used. The ratio of twitch torque production from the single twitch to the doublet was calculated as the criterion measure of LFF.

M-wave

The M-wave was measured before, during, and after all voluntary and involuntary tests. M-waves were recorded through surface EMG electrodes positioned with an interelectrode distance of 30 mm and a distance of more than 50 mm from the stimulating electrodes [34]. One pair of EMG electrodes was placed on the vastus lateralis, distally from the stimulating electrodes, and the other pair was placed on the

rectus femoris lateral to the stimulating electrodes. The reference was placed on the patella of the knee. Permanent ink was used to mark electrode placement between visits. EMG signals were collected at a sample rate of 2000 Hz, and were high and low pass filtered at 500 Hz and 10 Hz, respectively.

M-wave amplitudes were determined from the single twitches applied during the LFF assessments before, immediately after, and 3 minutes after each critical torque test. In addition, amplitudes were determined from the single twitches applied every 6th contraction for voluntary activation/ITT assessments during the voluntary test. During the stimulated critical torque tests, M-wave amplitude was determined by the application of single 1 ms twitches after every 3rd contraction during the first minute, and every 7th contraction during the final 4 minutes.

Interpolated twitch/voluntary activation

Voluntary activation and percent activation were determined during the voluntary test using the interpolated twitch technique. Approximately 2.5 seconds into the first voluntary contraction of the all-out test, a single-pulse stimulation was applied to determine the increase in torque above MVC (ITT). During the following relaxation phase, a single-pulse stimulation was applied (electrically evoked twitch; ETT). The twitch torques from the ITT and the EET were determined and used for analyses of muscle contractile properties. This method was used during all MVC assessments and every 6th contraction of the voluntary CT test. The following equation was used to determine percent activation (%act) [22]:

$$\%act = 100\% \times (1 - ITT/EET).$$

Blood lactate

Blood lactate concentrations was assessed during all exercise tests. Measures were made immediately before exercise, immediately after exercise, and 3 minutes post-exercise. The finger stick method was utilized for all measures.

3.4 Statistical analysis

Force values were converted to torque by multiplying force by each participant's moment arm. One-way repeated measures ANOVA were used to analyze the last 6 contractions (30 seconds) for the voluntary exercise protocol and the last 7 contractions (28 seconds) for each of the stimulated exercise protocols (100 Hz from the 2nd visit (100 Hz D1), 100 Hz from the 3rd visit (100 Hz D2), Intermediate frequency, and Below frequency) to determine whether a plateau in torque occurred during each exercise protocol. The average of the torque during the final 6 contractions of the voluntary test and the average of the torque during the final 7 contractions of each of the stimulated exercise tests were for end-test torque. Dependent t-tests were used to compare the mean absolute, the mean relative, the peak absolute, and the peak relative end-test torque to the first contraction of each of the exercise protocols. One-way repeated measure ANOVAs were used to analyze the percent activation during the voluntary exercise test, the mean absolute end-test torque, the mean relative end-test torque, the peak absolute end-test torque, and the peak relative end-test torque across the stimulated exercise protocols. One-way repeated measures ANOVAs were used to analyze the absolute and relative twitch torque for all exercise tests. LFF, lactate, and M-wave amplitudes from the rectus femoris and vastus lateralis will be compared using a 5 condition (voluntary, 100 Hz D1, 100 Hz D2, Intermediate frequency, and Below

frequency) x 5 time points (pre, first post exercise, average of the final 3 post exercise, average of the first 3 post rest, and the average of the final 3 post rest) repeated measured ANOVA. If there was an interaction, 1-way repeated measures ANOVAs were run to analyze differences across time and differences across conditions. Mauchly's test was used to determine assumptions of sphericity. If sphericity was violated, the Greenhouse-Geisser correction was applied. All statistical analysis was completed using SPSS 22 (IBM, Armonk, NY). Significance will be set at $p \leq 0.05$.

Protocol Overview for Voluntary CT Assessment

1. Blood lactate measure
2. LFF doublet/single twitch pairs x 10

   20 seconds
3. 60 MVC (3s exercise, 2s rest); ITT during every 6^{th} contraction

   2 seconds of rest
4. Blood lactate measure
5. LFF doublet/single twitch pairs x 10

   2 minutes of rest
6. Blood lactate measure
7. LFF doublet/single twitch pairs x 10

Protocol Overview of Stimulated CT Assessments

1. Blood lactate measure
2. LFF doublet/single twitch pairs x 10

   20 seconds

3. 75 involuntary contractions (2s exercise, 2s rest); M-wave pulses every 3rd contraction during the first minute and every 7th contraction thereafter

   2 seconds of rest
4. Blood lactate measure
5. LFF doublet/single twitch pairs x 10

   2 minutes of rest
6. Blood lactate measure
7. LFF doublet/single twitch pairs x 10

Chapter 4 – Results

4.1 Plateau in end-test torque

Force produced during the exercise protocols was expressed as absolute torque (Nm) and normalized as a percent change from MVC for the voluntary (VOL) protocol and as a percent change from the torque of the first contraction for the stimulated protocols (relative). Absolute and relative end-test torque (determined as the average of the final 6 contractions) differed significantly from the first contraction of VOL ($p < 0.001$).

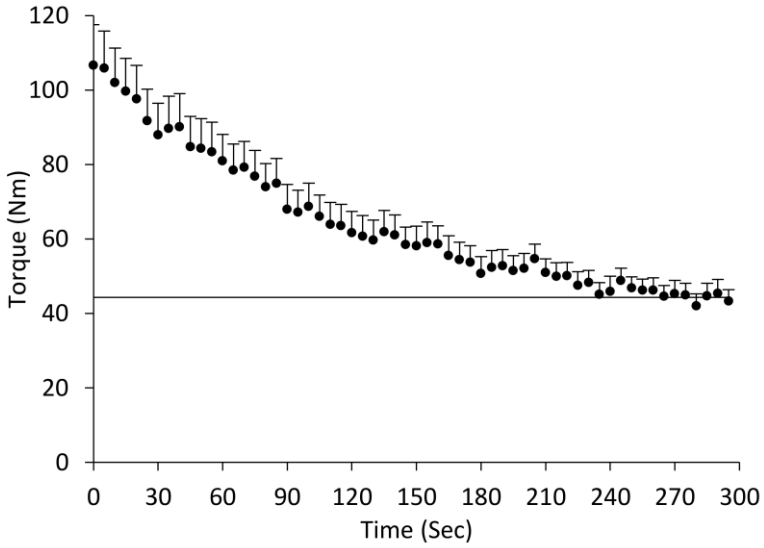
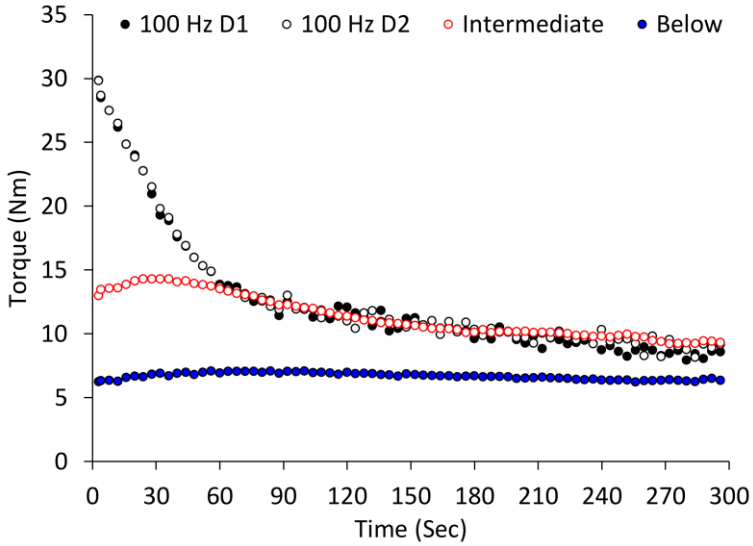


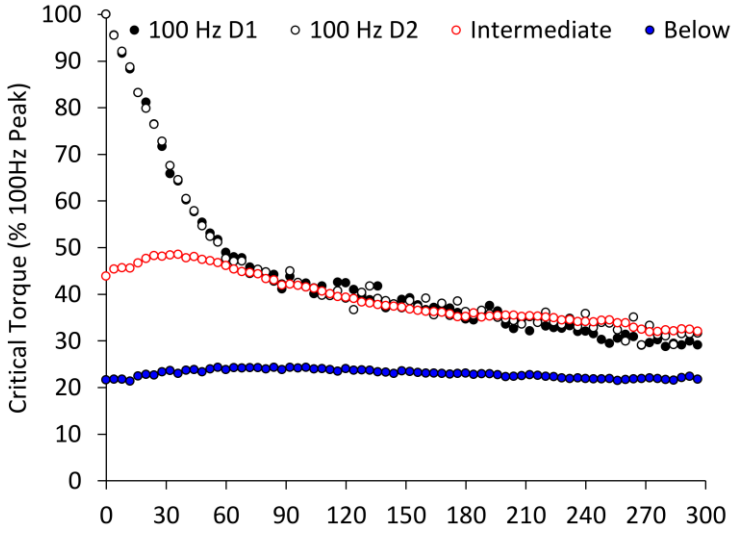
Figure 2 – Group peak torque during the 60 maximal voluntary contractions of the 5 minute protocol. Solid line indicates plateau in torque over final 6 contractions. Values are mean ± SEM.

Absolute and relative end-test torque (determined as the average of the final 7 contractions) differed significantly from the first contraction across the 100 Hz protocol of the first day (D1), the 100 Hz protocol of the second day (D2), and the Intermediate frequency (IF) exercise protocol ($p \leq 0.002$; Figure 3 and Figure 4). Absolute and peak

relative end-test torque of the low frequency (Below) exercise protocol did not differ significantly from the first contraction ($p \geq 0.558$; Figure 3 and Figure 4).



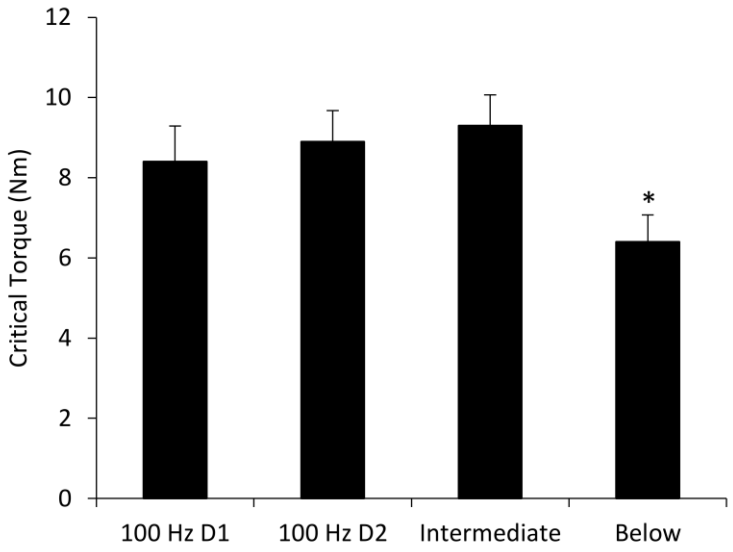
<u>Figure 3</u> – Group peak torque during the 75 stimulated contractions of each of the 5 minute stimulated protocols.



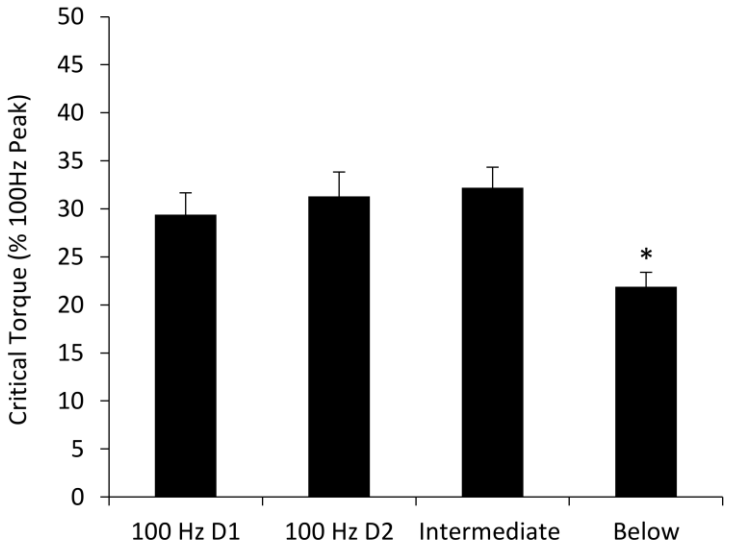
<u>Figure 4</u> – Group peak torque during the 75 stimulated contractions of each of the 5 minute stimulated protocols. All contractions are normalized to the starting torque achieved during the 100 Hz protocol.

4.2 End-test torque across exercise protocols

One-way repeated measures ANOVAs were used to compare the absolute and relative end-test torque values across the 4 stimulation protocols. Significant differences were found among the protocols ($p \leq 0.039$). Absolute (Figure 5), and relative (Figure 6) end-test torque did not differ significantly between the two 100 Hz visits ($p \geq 0.127$) or the IF protocol ($p \geq 0.133$). Absolute and relative end-test torque from the Below protocol differed significantly from the end-test torque of each of the other stimulated protocols ($p \leq 0.035$).



<u>Figure 5</u> – Peak critical torque values of each of the stimulated protocols. Critical torque is the mean of the final 7 contractions of each stimulated protocol. * indicates a significant difference from other protocols ($p \leq 0.05$). Values are mean ± SEM.



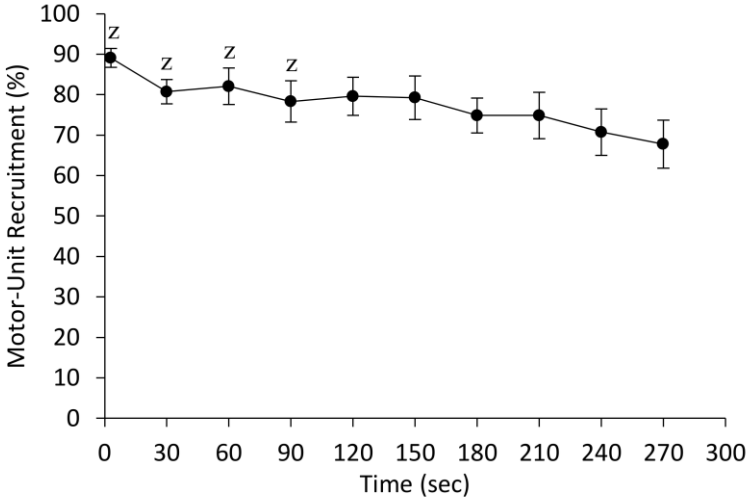
<u>Figure 6</u> – Peak critical torque values of each of the stimulated protocols normalized to starting torque achieved during the 100 Hz protocol. Critical torque is the mean of the final 7 contractions of each stimulated protocol. * indicates a significant difference from other protocols ($p \leq 0.05$). Values are mean ± SEM.

Mean and peak torque production during VOL was then expressed relative to the torque produced during the first contraction and compared to the relative end-test torques of the stimulation protocols. One-way ANOVAs were significant for both mean and peak torque ($p < 0.001$). Post hoc analysis indicated that relative mean and peak end-test torque during VOL were significantly higher than end-test torque during all of the stimulated exercise protocols ($p \leq 0.005$).

4.3 Voluntary motor unit recruitment

Motor-unit recruitment during the VOL protocol was expressed as a percentage. One-way ANOVA over time was significant ($p < 0.001$; Figure 7) with values for recruitment at Pre, 30-sec, 60-sec, and 90-sec into exercise being significantly higher than the value at the final assessment 270-sec into exercise ($p \leq 0.046$). Motor-unit

recruitment at time points 120-sec – 240-sec of exercise were not significantly different than the final measure ($p \geq 0.145$).



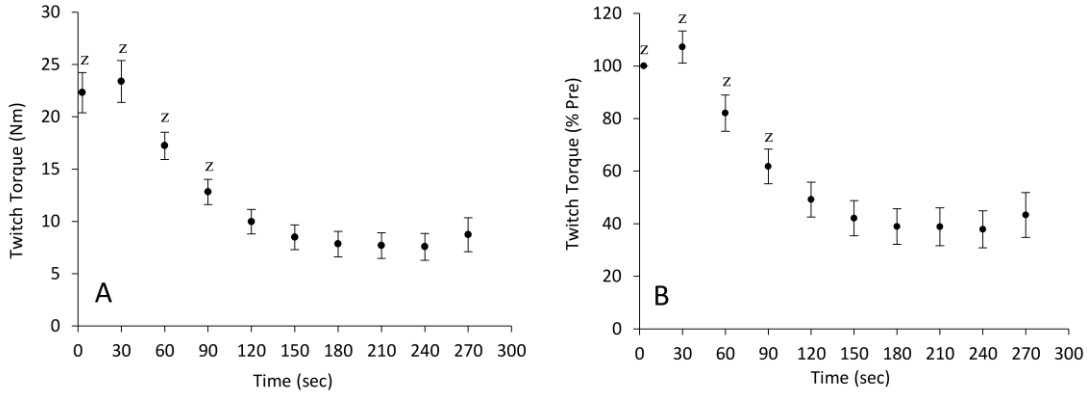
<u>Figure 7</u> – Motor-unit recruitment response during the voluntary protocol. ^{z} indicates a significant difference from value at 270 sec ($p \leq 0.05$). Values are mean ± SEM.

4.4 Twitch torque

Twitch torque during VOL was expressed as an absolute value (Nm) and as a percent change from the twitch torque produced during the highest MVC. Each stimulated exercise protocol was expressed as an absolute value (Nm) and as a percent change from average twitch torque of the final three twitches during the first low frequency fatigue assessment (Pre).

One-way ANOVAs over time for VOL were significant ($p < 0.001$). Post hoc analysis revealed that twitch torque measures from 60-sec – 270-sec were significantly lower than pre for both absolute torque ($p \leq 0.014$; Figure 8) and percent change ($p \leq$

0.022; Figure 8). By 120-sec, absolute and relative twitch torque plateaued and were not significantly different from the final measure ($p \geq 0.135$; Figure 8).



<u>Figure 8</u> – Twitch torque during the voluntary protocol as absolute twitch torque (A), and normalized to highest twitch torque achieved during MVC (B). ^{z} indicates a significant difference from value at 270-sec ($p \leq 0.05$). Values are mean ± SEM.

Mean twitch-torque values during the 4 stimulated protocols are shown in Figure 9. One-way ANOVAs for time during 100 Hz D1 were significant for both absolute and relative twitch torque ($p < 0.001$). Absolute twitch torques at Pre – 172-sec were significantly higher than the final time measure at 284-sec ($p \leq 0.002$). Time points 200-sec – 256-sec were not significantly different from 284-sec ($p \geq 0.06$). Pre – 228-sec were significantly different from 284-sec for relative twitch torque ($p \leq 0.028$). Relative twitch torque at 256-sec was not significantly different from 284-sec ($p = 0.11$).

One-way ANOVAs for time during 100 Hz D2 were significant for both absolute and relative twitch torque ($p < 0.001$). Absolute twitch torques at Pre – 172-sec were significantly higher than the final time measure at 284-sec ($p \leq 0.014$). Absolute twitch torque plateaued by 200-sec and remained plateaued for the remainder of the

protocol ($p \geq 0.096$). Relative twitch torques at Pre – 200-sec were significantly higher than the final time measure at 284-sec ($p \leq 0.018$). Relative twitch torque plateaued by 228-sec ($p \geq 0.33$).

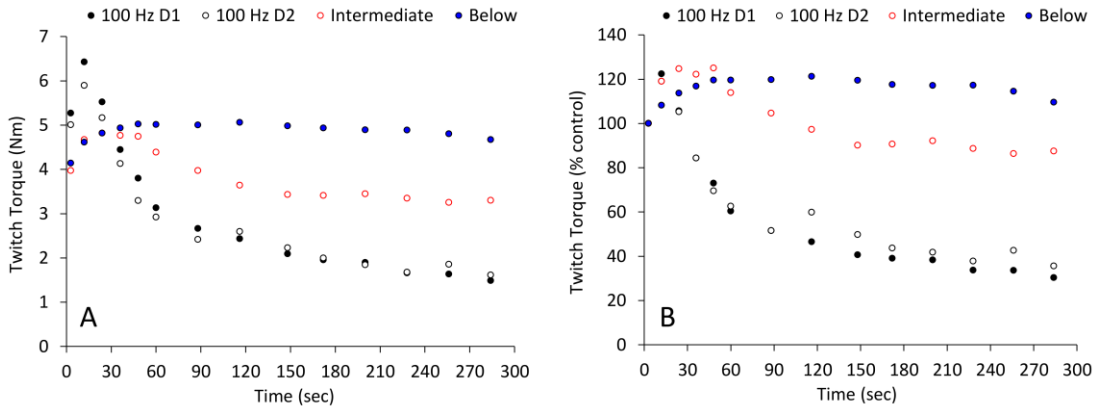
One-way ANOVAs for time during the IF protocol were significant for both absolute and relative twitch torque ($p \leq 0.002$). Absolute and relative twitch torque at Pre was not different from the final measure ($p \geq 0.076$). Absolute twitch torque from 12-sec – 116-sec was higher than the final measure at 284-sec ($p \leq 0.03$). By 148-sec, torque plateaued to a level similar to 284-sec ($p \geq 0.09$). Relative twitch torque from 12-sec – 148-sec was higher than the final measure at 284-sec ($p \leq 0.014$). By 172-sec, torque plateaued to a level similar to 284-sec ($p \geq 0.099$).

One-way ANOVAs for time during the Below protocol were significant for both absolute and relative twitch torque ($p \leq 0.037$). For absolute twitch torque, from 88-sec – 228-sec there was a transient, but significant, increase in torque compared to ending twitch torque ($p \leq 0.038$). All other time points were not different from the final measure ($p \geq 0.054$). For relative twitch torque, from 116-sec – 148-sec there was a similar, but briefer, elevation in torque compared to 284-sec ($p \leq 0.018$). All other time points were not different from ending twitch torque ($p \geq 0.052$).

Table 1. – Absolute and relative twitch torques during stimulated exercise

Absolute TT	**Pre**	**12-sec**	**24-sec**	**36-sec**	**48-sec**	**60-sec**	**88-sec**	**116-sec**	**148-sec**	**172-sec**	**200-sec**	**228-sec**	**256-sec**	**284-sec**
100 Hz D1	5.3 ± 2.6[z]	6.4 ± 3.3[z]	5.5 ± 2.8[z]	4.4 ± 2.4[z]	3.8 ± 2.0[z]	3.1 ± 1.7[z]	2.7 ± 1.5[z]	2.4 ± 1.3[z]	2.1 ± 1.1[z]	2.0 ±1.0[z]	1.9 ± 0.9	1.7 ± 0.7	1.6 ± 0.7	1.5 ± 0.7
100 Hz D2	5.0 ± 2.2[z]	5.9 ± 2.5[z]	5.2 ± 2.1[z]	4.1 ± 1.7[z]	3.3 ± 1.2[z]	2.9 ± 1.0[z]	2.4 ± 0.9[z]	2.6 ± 1.2[z]	2.2 ± 0.7[z]	2.0 ± 0.6[z]	1.8 ± 0.5	1.7 ± 0.4	1.9 ± 0.7	1.6 ± 0.7
Intermediate	4.0 ± 2.2	4.7 ± 2.6[z]	4.8 ± 2.5[z]	4.8 ± 2.5[z]	4.7 ± 2.2[z]	4.4 ± 2.1[z]	4.0 ± 1.9[z]	3.6 ± 1.8[z]	3.4 ± 1.8	3.4 ± 1.7	3.4 ± 1.7	3.3 ± 1.7	3.3 ± 1.7	3.3 ± 1.7
Below	4.1 ± 2.3	4.6 ± 3.1	4.8 ± 3.2	4.9 ± 3.2	5.0 ± 3.3	5.0 ± 3.3	5.0 ± 3.3[z]	5.1 ± 3.3[z]	5.0 ± 3.2[z]	4.9 ± 3.3[z]	4.9 ± 3.1[z]	4.9 ± 3.1[z]	4.8 3.1	4.7 ± 3.1
Relative TT														
100 Hz D1	100.0 ± 0.0[z]	122.5 ± 22.7[z]	105.7 ± 23.0[z]	84.4 ± 24.5[z]	73.0 ± 29.1[z]	60.5 ± 26.6[z]	51.6 ± 18.5[z]	46.6 ± 13.4[z]	40.7 ± 13.2[z]	39.1 ± 13.1[z]	38.4 ± 12.3[z]	33.7 ± 13.3[z]	33.6 ± 13.3	30.3 ± 10.5
100 Hz D2	100.0 ± 0.0[z]	119.1 ± 22.7[z]	105.3 ± 22.6[z]	84.4 ± 17.2[z]	69.6 ± 17.5[z]	62.7 ± 16.2[z]	51.6 ± 13.7[z]	59.9 ± 40.2[z]	49.8 ± 20.7[z]	43.7 ± 14.8[z]	41.8 ± 16.6[z]	37.9 ± 13.9	42.7 ±22.3	35.6 ±17.3
Intermediate	100.0 ± 0.0	119.1 ± 22.7[z]	124.7 ± 16.3[z]	122.2 ± 11.4[z]	125.0 ± 20.3[z]	113.9 ± 11.3[z]	104.6 ± 15.2[z]	97.3 ± 20.0[z]	90.2 ± 21.6[z]	90.6 ± 20.5	92.2 ± 25.2	88.7 ± 20.6	86.4 ± 19.9	87.5 ± 29.6
Below	100.0 ± 0.0	108.3 ± 18.6	113.7 ± 20.7	116.8 ± 21.0	119.6 ± 21.7	119.6 ± 22.1	119.8 ± 21.6	121.3 ± 21.6[z]	119.5 ± 21.8[z]	117.6 ± 22.1	117.1 ± 21.1	117.3 ± 21.6	114.5 ± 22.0	109.6 ± 24.1

Values are mean ± SD
[z] indicates a significant difference from Pre



<u>Figure 9</u> – Twitch torque during the stimulated protocols as absolute twitch torque (A), and normalized to mean of final 3 twitches during the LFF assessment (B).

4.5 Low frequency fatigue

Low frequency fatigue (LFF) was expressed as the ratio of the torque produced by a single twitch to a doublet twitch as well as the percent change from the average of last 3 single/doublet pairs during the pre exercise assessment of LFF (Pre). LFF was assessed immediately post exercise (IP), 45 seconds post exercise (45-sec), 180 seconds post exercise (180-sec), and 230 seconds post exercise (230-sec). There was a significant protocol x time interaction for both LFF ratios and percent change measures ($p < 0.001$). One-way repeated measures ANOVAs were run for each exercise protocol and for each time point post exercise.

One-way repeated measure ANOVAs across time for VOL was significant for both ratio and percent change ($p < 0.001$; Figure 10 and 11). LFF was significantly reduced from pre exercise measures at all post exercise time points for both ratio and percent change values ($p \leq 0.004$).

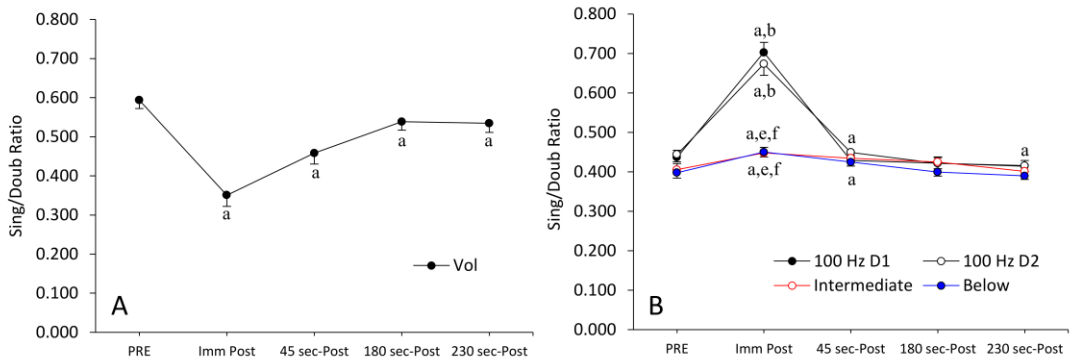
For D1, 1-way repeated measures ANOVAs were run for both ratio and percent change. Both were significant ($p < 0.001$). The single/doublet ratio was higher at IP

than at all other time points for both ratio and percent change values ($p < 0.001$). Ratio and percent change values returned to pre levels by 45 s and no other times points were significantly different from pre exercise ($p \geq 0.22$).

One-way repeated measures ANOVAs for ratio and percent change for D2 were completed ($p < 0.001$). The single/doublet ratio was significantly higher at IP than all other time points for both ratio and percent change values ($p < 0.001$). In addition, the ratio at 230 s was significantly lower than pre ($p = 0.045$). All other ratio and percent change values had returned to pre levels by 45 s ($p \geq 0.06$).

For IF, 1-way repeated measures ANOVAs were run for both ratio and percent change. Both were significant ($p \leq 0.010$). Ratio and percent change values of LFF were higher at IP and 45 s ($p \leq 0.040$). LFF ratio and percent change eventually dropped to pre exercise levels by 180 s ($p \geq 0.13$).

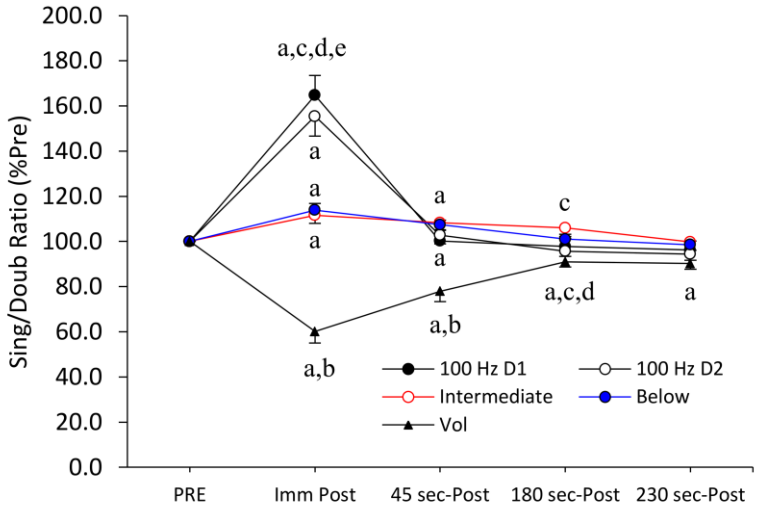
For Below, 1-way repeated measures ANOVAs were run for both ratio and percent change. Both were significant ($p < 0.001$). Ratio and percent change values were higher at IP and 45 s ($p \leq 0.004$). Ratio and percent change eventually dropped to pre exercise levels by 180 s ($p \geq 0.27$).



<u>Figure 10</u> – Single/doublet ratio pre and post voluntary protocol (A), and pre and post stimulated protocols (B). [a] indicates a significant difference from pre value. [b] indicates significant difference from all other stimulated protocols. [c] indicates significant difference from the Intermediate protocol. [d] indicates significant difference from the Below frequency protocol. [e] indicates significant difference from 100 Hz D2 protocol. [f] indicates significant difference from 100 Hz D1 protocol ($p \leq 0.05$). Values are mean ± SEM.

There was a main effect for protocol at IP for ratio and percent change ($p < 0.001$). Ratio and relative LFF during VOL was lower than each of the stimulated exercise tests ($p \leq 0.015$). The ratio and relative LFF values for D1 and D2 were higher than the other tests ($p < 0.001$), with D1 being higher than D2 ($p \leq 0.050$). IF and Below were not different from each other at IP for either ratio or relative ($p \geq 0.64$). There was not a main effect for protocol at 45-sec for ratio LFF ($p = 0.37$). There was a main effect for protocol at 45-sec for relative LFF ($p < 0.001$). However, by 45-sec, percent change in LFF for both D1 and D2 decreased to similar values as IF and Below ($p \geq 0.14$). Percent change in LFF during VOL remained suppressed compared to all of the stimulated tests ($p \leq 0.010$). There was also a main effect for protocol at 180-sec for ratio and percent change ($p \leq 0.009$). Ratio LFF during VOL was significantly higher than LFF for all of the stimulated tests ($p < 0.001$). Ratio LFF was not significantly different between any of the stimulated tests ($p \geq 0.116$). At 180-sec, VOL remained

lower than IF and Below for percent change ($p \leq 0.006$). D2 was also significantly lower than IP for percent change ($p = 0.032$). There was a main effect for protocol at 230-sec for ratio LFF ($p < 0.001$). Ratio LFF during VOL was significantly higher than LFF for all of the stimulated tests ($p < 0.001$). Ratio LFF was not significantly different between any of the stimulated tests ($p \geq 0.09$). By 230-sec, there was no main effect for protocol for percent change ($p = 0.19$).



<u>Figure 11</u> - Single/doublet ratio pre and post stimulated protocols normalized to pre. [a] indicates a significant difference from pre value. [b] indicates significant difference from all stimulated protocols. [c] indicates significant difference from the Intermediate protocol. [d] indicates significant difference from the Below frequency protocol. [e] indicates significant difference from 100 Hz D2 protocol ($p \leq 0.05$). Values are mean ± SEM.

4.6 Vastus lateralis M-wave

All vastus lateralis (VL) M-wave values were expressed relative to the average of the 10 M-waves collected during the pre-exercise LFF protocol (Pre). Changes in VL M-wave over time during exercise can be seen in Figure 12A. In order to compare the changes that occurred in M-wave among the exercise protocols only VL M-wave assessed Pre, immediately post (IP), 45 seconds post (45-sec), 180 seconds post (180-

sec), and 230 seconds post (230-sec) exercise were statistically compared. There was a significant protocol x time interaction for relative VL M-wave ($p = 0.007$; Figure 12B).

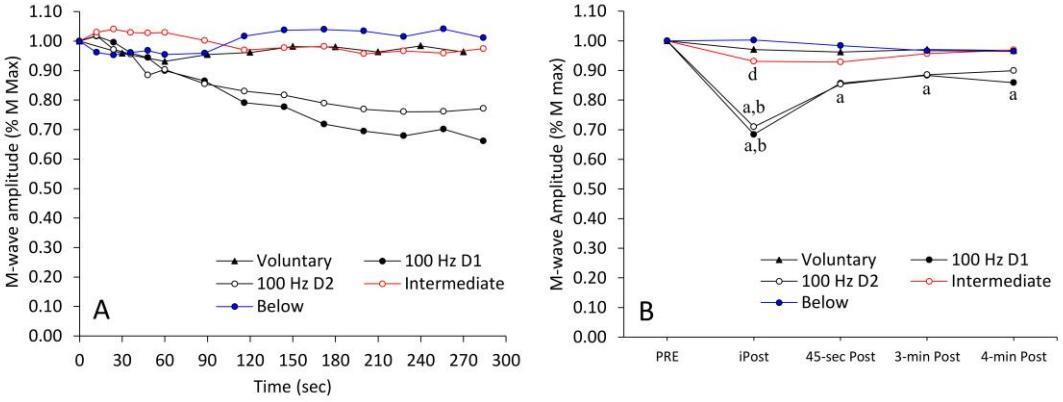
A one-way repeated measures ANOVA during VOL was not significant ($p = 0.19$; Figure 12B), indicating no changes to M-wave between pre, IP, 45-sec, 180-sec, and 230-sec. There was a significant effect over time during D1 ($p = 0.004$; Figure 12B). During D1, M-wave was significantly lower than pre at all post exercise measures ($p \leq 0.025$). Despite never recovering to Pre values, M-wave partially recovered to the extent that 45-sec, 180-sec, and 230-sec were not significantly different from each other ($p \geq 0.13$).

Likewise, there was a significant effect for time for D2 ($p = 0.024$; Figure 11). Unlike D1, during D2, M-wave was only significantly lower than pre at IP ($p = 0.027$). M-wave recovered by 45-sec, and was not significantly different from pre at any other measure ($p \geq 0.058$). IP was significantly lower from all of the subsequent post measures ($p \leq 0.020$). The final three post exercise measures were not significantly different from each other ($p \geq 0.17$).

One-way ANOVAs for time for both IF and Below were not significant ($p \geq 0.197$; Figure 12B), indicating that M-wave did not significantly increase or decrease at any time point during those protocols.

There was a significant effect for protocol at the IP time point ($p = 0.009$; Figure 12B). M-wave amplitude during VOL was not significantly different than IF or Below ($p \geq 0.16$), but amplitude at IF was lower than Below ($p = 0.050$). M-wave amplitudes at D1 and D2 were not significantly different from each other ($p = 0.835$), but were

significantly lower than the other 3 protocols ($p \leq 0.045$). There was not an effect for protocol at 45-sec, 180-sec, and 230-sec ($p \geq 0.068$).



<u>Figure 12</u> – Vastus lateralis M-wave amplitude during voluntary and stimulated protocols (A), and pre and post protocols (B). All amplitudes are normalized to the mean of the 10 M-wave amplitudes induced during the LFF assessment before exercise. [a] indicates a significant difference from pre value. [b] indicates significant difference from all stimulated protocols. [d] indicates significant difference from low frequency protocol ($p \leq 0.05$). Values are mean ± SEM.

4.7 Lactate

There was a significant protocol x time interaction for blood lactate values taken at rest (pre), immediately post (IP), and 3 minutes post (3P) exercise ($p < 0.001$; Figure 13). One-way repeated measures ANOVAs were run for each exercise protocol and for each time point.

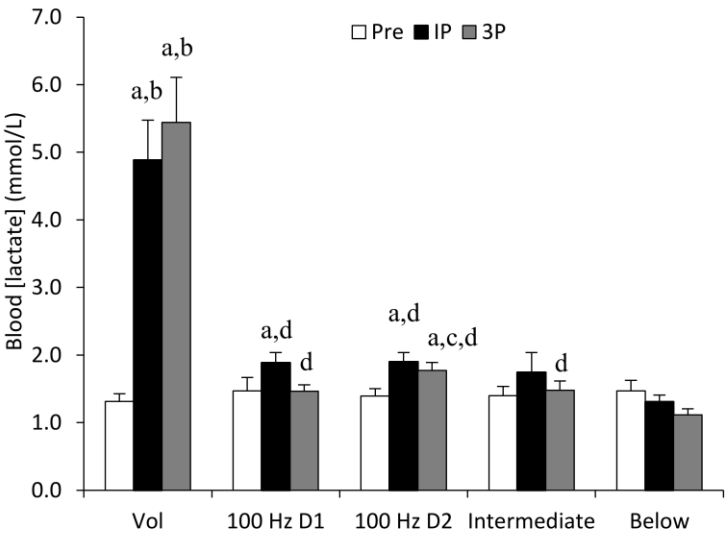
One-way ANOVA demonstrated that resting lactate levels were not different between exercise protocols ($p = 0.843$). There was a main effect for protocol at both IP and 3P ($p < 0.001$). Lactate levels IP and 3P during the voluntary protocol were significantly higher than the IP and 3P measures of all of the stimulated protocols ($p \leq 0.001$). D1 and D2 were significantly higher than the low frequency test at both IP and 3P measures ($p \leq 0.018$). D1 and D2 were not significantly different from the

Intermediate frequency protocol at IP ($p \geq 0.59$), but D2 was significantly higher than Intermediate at 3P ($p = 0.044$), while D1 was not ($p = 0.598$). Lactate during IF was not significantly higher than Below at IP ($p = 0.234$), but was higher at 3P ($p = 0.017$).

There was a main effect for time during VOL ($p < 0.001$). Lactate increased in response to VOL as IP was significantly higher than resting ($p < 0.001$). Lactate levels continued to rise during the rest period after exercise, and were significantly higher at 3P than IP ($p = 0.017$).

There was a main effect for time for D1 and D2 ($p \leq 0.035$). Lactate increased above resting levels immediately after D1 ($p = 0.022$). During the resting period, lactate levels decreased so that at 3P, lactate was lower than IP ($p = 0.003$) and back to resting levels ($p = 0.97$). Similar to D1, D2 lactate increased in response to the exercise protocol ($p = 0.004$). However, lactate levels remained elevated during the 3 min rest period so that IP and 3P measures were not different ($p = 0.34$), and 3P measures were higher than resting levels ($p = 0.010$).

One-way ANOVAs across time were not significant for the Intermediate and Below frequency protocols ($p \geq 0.16$), indicating that lactate did not change during these tests.



<u>Figure 13</u> – Blood lactate concentration responses before, immediately after, and 3 minutes after voluntary and stimulated exercise protocols. [a] indicates significant difference from pre. [b] indicates significant difference from all stimulated protocols. [c] indicates significant difference from Intermediate protocol. [d] indicates significant difference from the Below frequency protocol ($p \leq 0.05$). Values are mean ± SEM.

Chapter 5 – Discussion

Critical power/torque represents a threshold of exercise output or intensity. Hypothetically, exercise below that intensity can be maintained indefinitely, as VO_2 and metabolite production should reach a steady-state. Above the critical work-rate threshold, VO_2 increases and metabolites continue to build-up, ultimately resulting in exercise intolerance. This critical threshold phenomenon and its ability to predict time to fatigue is well studied under voluntary conditions, and can be expressed using the following equation [6]:

$$T_{lim} = W' / (P - CP)$$

Despite several studies [18, 19] demonstrating what appears to be a similar hyperbolic time-duration relationship under electrically stimulated conditions, the critical threshold concept has not yet been applied to involuntary exercise. Studying the power-duration relationship under stimulated conditions would allow for the removal of the voluntary aspect, potentially isolating the peripheral fatigue mechanisms that are contributing the stabilization of work output. The purpose of this current study was 1) to observe if an electrically stimulated exercise protocol in the quadriceps results in a hyperbolic power-duration pattern seen in voluntary contractions; and 2) determine if the decline in torque production over time during electrically stimulated exercise occurred due to similar mechanism(s) as the decline in torque during voluntary exercise. Ultimately, we hoped to determine if a critical torque test using neuromuscular electrical stimulation would be possible and useful for further studies.

5.1 Critical torque

We found that relative torque of the voluntary protocol plateaued at approximately 40% of the maximal voluntary contraction, which is very similar to the

findings of others [14] using an identical protocol in the knee extensors. Relative torque decreased and then plateaued at approximately 30% of starting torque during both D1 and D2 of 100 Hz exercise and during the Intermediate frequency exercise protocol. These findings likely indicate the attainment of a metabolic steady-state or homeostasis. The attainment of a steady-state in response to electrically stimulated exercise are similar to the findings of other studies [18]. The 100 Hz tests declined approximately 70% from starting torque, while torque declined approximately 25% during the Intermediate protocol—although it plateaued at a similar absolute torque level compared to D1 and D2 of 100 Hz stimulation. Similar absolute and relative end-test torque values between the two 100 Hz tests demonstrate the consistency of this torque value, providing further support of a critical threshold that is similar from day-to-day. Our hypothesis was partially supported by the end-test torques of the Intermediate and one of the 100 Hz tests being similar. Interestingly, when exercise was performed using a stimulation frequency that elicited a starting torque that was lower than the end-test torque during the 100 Hz and Intermediate protocols, no change in torque (i.e. no fatigue occurred) was observed during exercise. This finding further supports the supposition that the end-test torque observed during 100 Hz and Intermediate frequency stimulation represents a "critical" threshold where a metabolic steady-state occurs. These findings support our hypotheses that end-test toque during stimulated exercise represents a similar parameter to the CT parameter observed during voluntary exercise.

5.2 Fatigue Mechanisms

Voluntary activation

Since voluntary activation describes how much muscle can be activated during voluntary efforts, declines in motor unit recruitment are indicative of generalized central fatigue [14, 22]. Voluntary activation of motor units declined to approximately 70% by the end of the voluntary exercise protocol, similar to the decline observed previously using the 5 minute all out test [14]. Taken together these findings clearly demonstrate a central fatigue component to the attainment of critical torque under voluntary exercise conditions. Work by Amann [2, 9-12] has shown an interaction between the accumulation of metabolic by-products and subsequent activation of type III and type IV afferent nerve fibers during exercise and a decline in the ability of the CNS to activate/recruit motor-units. Thus our findings of a decline in %ACT during the CT test may be the result of type III and type IV afferent nerves inhibiting voluntary motor-output, rather than a decline in effort from our participants.

Twitch torque

Declines in twitch torque are generally thought to be indicative of peripheral fatigue. Twitch torque dropped approximately 60% during the voluntary test, comparable to the decline in MVC torque. Twitch torque plateaued halfway through the test, long before MVC torque plateaued, indicating that peripheral fatigue was a contributor to declines in MVC during the first half of the critical torque test, but less so during the latter half of the test. Likewise, during the 100 Hz exercise protocol, twitch torque declined to a similar percentage of starting torque as the stimulation trains – about a 70% decline. However, twitch torque did not plateau until approximately 200 seconds.

Interestingly, twitch torque during the Intermediate frequency test did not fall to a similar percentage of starting torque as did torque during the stimulation trains. While torque declined 25% during the trains, there was only a 13% decline in twitch torque. This discrepancy suggests there may be different fatigue mechanisms affecting torque production during stimulation trains, which last multiple seconds and contain many individual twitches, and during a single twitch. This discrepancy may be due to differences in changes in sarcolemma excitability, t-tubule excitability, and/or calcium release from the sarcolemma.

Low frequency fatigue

There was significant low frequency fatigue during/following the voluntary critical torque test. While there were signs of recovery during the 4 minutes of rest following the test, the single/doublet ratios did not return to resting levels. This is expected, as low frequency fatigue following certain types of voluntary exercise has been shown to persist for minutes, hours, or days [21, 48]. LFF is thought to represent impairment of calcium release/re-uptake by the sarcoplasmic reticulum—therefore it is likely that some of the decline in torque production that occurred during the voluntary CT test can be attributed to peripheral fatigue due to impair calcium kinetics.

Surprisingly, we observed acute high frequency fatigue following both 100 Hz protocols. Immediately after the conclusion of the exercise protocol, doublet torque levels were suppressed to the point that they nearly matched by the torque produced by a single twitch. This high frequency fatigue was transient, as the ratios returned to pre exercise levels by 45-sec post exercise. The Intermediate and Below frequency stimulation tests also produced high frequency, although to a much lesser extent than

the high frequency test. This effect was also short-term, as the ratios returned to normal by 45-sec post exercise. The mechanism(s) responsible for this observation are unclear, but could potentially be related to depletion of acetylcholine at the neuromuscular junction or impairments in repolarization of the sarcolemma and/or t-tubules that limit the transmission of the second action potential during the doublet stimulation.

M-wave

A decline in M-wave amplitude during exercise is indicative of neuromuscular transmission failure, which can be caused by reduced transmission of action potentials along the muscle sarcolemma, or, potentially, depletion of acetylcholine release at the neuromuscular junction [31]. Takata and Ikata observed a 75% decline in M-wave amplitude during the first 4 minutes of direct nerve stimulation of rat gastrocnemius muscle using a 1.5 s : 0.5 s duty cycle at 100 Hz [31]. In our 5 minute 100 Hz protocols, we observed a 20-30% decline in M-wave amplitude in the vastus lateralis, indicating some transmission failure occurred. The difference in M-wave decline between the study of Takata and Ikata and ours is mostly likely related to the use of nerve stimulation, which is known to be more fatiguing, and the 3:1 stimulation work-to-rest cycle compared to our use of a 1:1 work-to-rest cycle. Allowing greater rest between subsequent contractions should allow for greater acetylcholine re-uptake at the motor-end plate and allow for greater repolarization in the sarcolemma. Takata and Ikata speculated that the magnitude of neuromuscular transmission failure may be proportional to the frequency of stimulation [31]—with higher frequencies of stimulation resulting in greater transmission failure. Our data support this hypothesis as

reducing the stimulation frequency during the Intermediate and Below protocols resulted in no changes in m-wave magnitude.

During the voluntary CT test, M-wave magnitude did not change—similar to our findings from the Intermediate and Below stimulation protocols. This finding suggests that neuromuscular transmission failure was likely not responsible for any of the peripheral fatigue observed during the voluntary CT test. Our M-wave data highlights the inherent differences between stimulated contractions/exercise and voluntary exercise. Stimulated contractions only recruit a portion of the motor-units of a given muscle and the same motor-units are recruited repeatedly during stimulated contractions [25]. This typically leads to greater fatigue and fatigue caused by different mechanisms than what is observed during voluntary exercise.

Lactate

Lactate increased significantly more as a result of the voluntary exercise protocol than from any of the stimulated exercise protocols. Since the amplitude of stimulation used during the involuntary tests was only high enough to elicit 25% of the voluntary MVC, less muscle was recruited. Since only a portion of the muscle was recruited, there was less total metabolic demand compared to the voluntary test.

Lactate increased significantly as a result of the high frequency stimulation (100 Hz), but not as a result of the Intermediate or Below frequencies. This indicates that the higher frequency was more metabolically demanding, and that the Intermediate frequency does not replicate the metabolic demand of the voluntary critical torque test.

5.3 Experimental Considerations

Given the differences in motor unit recruitment patterns between voluntary and stimulated conditions, extreme caution must be taken to avoid making direct comparisons between the two methods [25]. We must also consider that electrical stimulation only activates a small percentage (~25%) of the quadriceps muscles, while a maximal voluntary contraction can activate much closer to 100%.

Voluntary exercise was performed with a 60% duty cycle (3 s contraction, 2 s rest), while stimulated exercise was performed with a 50% duty cycle (2 s contraction, 2 s rest). It is very likely that fatigue characteristics were influenced by these differences [6]. However, pilot testing indicated that using high frequencies for 3 s induced muscle rolling and spasms that likely altered the area of stimulated muscle. In order to reduce the possibility changing muscle recruitment, we chose to use a shorter duty cycle for the stimulation tests in this current study.

Participants completed two stimulated exercise tests per visit. As a result, it is possible that there was some residual low frequency fatigue at the start of the second stimulation test. However, since the stimulated tests resulted in high frequency fatigue, but not low frequency fatigue, it is not likely that there was any residual effect.

5.4 Conclusions

Our findings support the general hypothesis that intermittent electrically stimulated isometric exercise leads to a steady-state end test torque, and it is similar among different frequencies of stimulation, despite dissimilar patterns of fatigue (neuromuscular transmission failure, Ca2+ release, metabolic demand, etc.). This threshold appears to be reproducible from day to day. More testing is necessary to see if there is some common factor that could explain the similar end-test torque.

There were apparent differences in fatigue mechanisms between voluntary and electrically stimulated conditions. Peripheral fatigue under voluntary conditions was likely driven by calcium kinetics and afferent feedback, while fatigue under stimulated conditions was driven by decreases in sarcolemma excitability. Despite these differences, testing critical torque under stimulated conditions could still be a useful tool for assessing fatigue profiles of both athletic and clinical populations.

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APPENDIX A: IRB APPROVAL LETTER

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Approval of Study Modification – Expedited Review – AP0

Date: February 27, 2017 **IRB#:** 6885

Principal Investigator: Christopher D Black **Reference No:** **663017**

Study Title: Estimation of critical torque using neuromuscular electrical stimulation of the quadriceps in humans.

Approval Date: 02/27/2017

Modification Description:
Adding Jessica Peterson as key study personnel

The review and approval of this submission is based on the determination that the study, as amended, will continue to be conducted in a manner consistent with the requirements of 45 CFR 46.

To view the approved documents for this submission, open this study from the My Studies option, go to Submission History, go to Completed Submissions tab and then click the Details icon.

If the consent form(s) were revised as a part of this modification, discontinue use of all previous versions of the consent form.

If you have questions about this notification or using iRIS, contact the HRPP office at (405) 325-8110 or irb@ou.edu. The HRPP Administrator assigned for this submission: Karen Braswell.

Cordially,

Lara Mayeux, Ph.D.
Chair, Institutional Review Board

APPENDIX B: INFORMED CONSENT FORM

Signed Consent to Participate in Research

Would you like to be involved in research at the University of Oklahoma?

I am Dr. Christopher Black from the Department of Health and Exercise Science, and I invite you to participate in my research project entitled "Estimation of Critical Torque Using Neuromuscular Electrical Stimulation of the Quadriceps in Humans." This research is being conducted at the Sensory and Muscle Function Laboratory. You were selected as a possible participant because you have no history of lower leg injuries, have no contraindications to performing resistance exercise, and are not pregnant. You must be between the ages of 18 and 45 to participate in this study.

Please read this document and contact me to ask any questions that you may have BEFORE agreeing to take part in my research.

What is the purpose of this research? The purpose of this research is to determine the effects of neuromuscular electrical stimulation on force output during isometric exercise.

How many participants will be in this research? About 30 men and women will take part in this research.

What will I be asked to do? If you agree to be in this research, you will be asked to visit the lab 5 times. You will complete 2 familiarization visits and 3 testing visits. During the testing visits, there will be an additional number of contractions that will not be performed during the familiarization. The purpose of the familiarization visits is to introduce you to the methods that will be used during the testing visits. During the familiarization visits, you will be seated in an adjustable chair, with your leg strapped to a lever arm. Then, we will place electrodes over the vastus lateralis muscle and the vastus medialis muscle of the quadriceps. There are four muscles in the quadriceps, and the vastus lateralis is the outermost muscle of the group and the vastus medialis is the innermost muslce of the group. We will complete a test called a current determination, in which we apply a series of electrical currents to your quadriceps so that they contract without you voluntarily contracting them. Each of the pulses will be 1 millisecond in length. Each application of electrical current can be thought of as a "shock" and allows us to make measures of the amount of force that your quadriceps muscle group can generate. This "shock" can be compared to the feeling of someone slapping or lightly punching your leg. We will gradually increase the intensity of the stimulation until there is a plateau in force produced, or until it is no longer comfortable for you. You will be asked to complete 3 maximal voluntary isometric contractions (MVC, exercises where you contract your quadriceps muscles by kicking out as hard as you can, but they do not move because you are pushing against padded straps). We will apply electrical stimulation approximately 2.5 seconds into each MVC, and then again 1 second after.

Each pulse will last 1 millisecond. We will measure the force produced by the contractions. You will then complete a shortened version of the voluntary test that you will complete during the testing visits. You will be asked to kick as hard as you can in increments of 3 seconds, followed by 2 seconds of rest. You will complete 36 of these repeated maximal contractions, and the test will last 3 minutes. You will be cued to kick and relax by a metronome and by verbal instruction. You will be encouraged to kick as hard as you can for the duration of the test despite inevitable drops in torque. During the second familiarization visit only, after the 3 individual MVCs and before the practice voluntary test, we complete a second current determination test, in which we will apply another series of electrical currents to your quadriceps. This time, each stimulation train will last 2 seconds. We will gradually increase the intensity of the stimulation until the force produced equals ~25% of your MVC, or until it is no longer comfortable for you. Then you will receive constant frequency stimulation trains at that intensity. The frequency will start at 5 Hz and will increase in increments of 5 Hz until reaching 100 Hz. We will use the force produced from each of these contractions to create a force-frequency curve.

During the testing visits, we will place the electrodes in the same place as during the familiarization visits. You will complete the two current determination tests that were completed in the familiarization visits. You will complete the 3 MVCs with the electrical stimulation twitches during and after. During one of the testing visits, you will be asked to complete a 5-minute all-out voluntary critical torque test. You will kick as hard as you can in increments of 3 seconds, followed by 2 seconds of rest. You will complete 60 of these repeated maximal contractions, and the test will last 5 minutes. You will be cued to kick and relax by a metronome and by verbal instruction. You will be encouraged to kick as hard as you can for the duration of the test despite inevitable drops in torque. During this voluntary critical torque test, electrical stimulation twitches will be applied during and immediately after every 6^{th} contraction (every 30 seconds). Before and after the critical torque test, low frequency fatigue (LFF) and M-wave amplitudes will be assessed by applying a series of doublets (two 1-millisecond pulses spaced 6 milliseconds apart) and single twitches. Each doublet will be paired with a single twitch spaced 3 seconds apart, and each pair will be spaced 3 seconds apart. Before the critical torque test, participants will receive 10 doublet/single stimulation pairs, and the critical torque test will be initiated 20 seconds after the final twitch. Two seconds after the conclusion of the critical torque test, 10 more doublet/single pairs will be applied. Another 10 pairs will be applied 2 minutes later. The current that was determined using the 1 millisecond twitches will be used for this assessment. Blood lactate will be measured by using a finger stick immediately before the exercise test, immediately after the test, and 3 minutes after the test.

During another testing visit, you will complete a stimulation test that will not require any active participation on your part. You will be asked to remain as relaxed as possible and to try not to contract your quadriceps muscles during the test. During the test, an

electrical current will be applied to the quadriceps muscles at a frequency of 100 Hz. The current will be applied in increments totaling 4 seconds, with 2 seconds "on" and 2 seconds "off", for a total of 75 increments (5 total minutes). Pulse duration will be held constant at 200 microseconds and amplitude will be at a level sufficient to evoke 25% of MVC at 100 Hz. We will measure the torque produced by each of the individual contractions completed during each test. Current amplitude will be held constant for the duration of the stimulation test. During this involuntary critical torque test, electrical stimulation twitches will be applied immediately after every 3rd contraction during the first minute and every 7th contraction thereafter. Before and after the critical torque test, low frequency fatigue (LFF) and M-wave amplitudes will be assessed by applying a series of doublets (two 1-millisecond pulses spaced 6 milliseconds apart) and single twitches. Each doublet will be paired with a single twitch spaced 3 seconds apart, and each pair will be spaced 3 seconds apart. Before the critical torque test, participants will receive 10 doublet/single stimulation pairs, and the critical torque test will be initiated 20 seconds after the final twitch. Two seconds after the conclusion of the critical torque test, 10 more doublet/single pairs will be applied. Another 10 pairs will be applied 2 minutes later. The current that was determined using the 2 second trains will be used for these assessments. Blood lactate will be measured by using a finger stick immediately before the exercise test, immediately after the test, and 3 minutes after the test.

After 30 minutes of rest, you will complete a second stimulation test, in the same manner as described above. However, the frequency of stimulation for this test will be determined by examining your force-frequency curve and the end-test torque from your first stimulation test. The frequency applied during this stimulation test will elicit a torque level that is below the end-test torque produced at 100 Hz. M-wave pulse, LFF, and blood lactate will be assessed in a similar manner.

The other testing visit will be similar, with exception of the firing frequencies for the 5-minute stimulation tests. The first test will be at a frequency that elicits a torque level that equals approximately 50% of the peak torque minus the end-test torque from the 100 Hz test. The second test will be at 100 Hz.

How long will this take? Your participation will take 5 visits, each lasting approximately 2 hours. The second visit will take place at least 24 hours after the first visit, and the following visits will take place at least 48 hours apart. The total time commitment for this study is approximately 10 hours.

What are the risks and/or benefits if I participate? During the exercise protocols, an electrical current will be applied to the vastus lateralis and vastus medialis muscles of your quadriceps. You may experience pain and/or discomfort in your quadriceps from the electrical stimulation and the force of the contractions. The intensity of pain or discomfort varies from person to person. It may gradually progress to the sensation similar to the stinging feeling in your hand after performing a very hard "high five". There is minimal risk of developing muscle soreness or injury resulting from isometric exercise. There are no direct benefits to participating in this study.

What do I do if I am injured? If you are injured during your participation, report this to a researcher immediately. Emergency medical treatment is available. However, you or your insurance company will be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

Will I be compensated for participating? You will be given a $10 gift card for completing the study.

Who will see my information? In research reports, there will be no information that will make it possible to identify you. Research records will be stored securely and only approved researchers and the OU Institution Review Board will have access to the records.

You have the right to access the research data that has been collected about you as a part of this research. However, you may not have access to this information until the entire research has completely finished and you consent to this temporary restriction.

Do I have to participate? No. If you do not participate, you will not be penalized or lose benefits or services unrelated to the research. If you decide to participate, you don't have to answer any question and can stop participating at any time.

Will my identity be anonymous or confidential? Your name will not be retained or linked with your responses. The data you provide will be destroyed unless you specifically agree for data retention or retention of contact information at the end of the research. Please check all of the options that you agree to:

I agree for the researcher to use my data in future studies. ___Yes ___ No

Photographing of Research Participants/Activities In order to preserve an image related to the research, photographs may be taken of participants. These photos may be used for research publication or for posters. You have the right to refuse to allow photographs to be taken without penalty. Please select one of the following options:

I consent to photographs. ___ Yes ___ No

Will I be contacted again? The researcher would like to contact you again to recruit you into this research or to gather additional information.

_____ I give my permission for the researcher to contact me in the future.

_____ I do not wish to be contacted by the researcher again.

Who do I contact with questions, concerns or complaints? If you have questions, concerns or complaints about the research or have experienced a research-related injury, contact me at 918-293-8976 or nataliejanzen@gmail.com. Additionally, you may contact Dr. Christopher Black at 405-325-7668 or cblack@ou.edu.

You can also contact the University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu if you have questions about your rights as a research participant, concerns, or complaints about the research

and wish to talk to someone other than the researcher(s) or if you cannot reach the researcher(s).

You will be given a copy of this document for your records. By providing information to the researcher(s), I am agreeing to participate in this research.

Participant Signature	Print Name	Date
Signature of Researcher Obtaining Consent	Print Name	Date
Signature of Witness (if applicable)	Print Name	Date

APPENDIX C: HIPAA

AUTHORIZATION TO USE or SHARE HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH

An Informed Consent Document for Research Participation may also be required.

Title of Research Project: **Estimation of Critical Torque Using Neuromuscular Electrical Stimulation of the Quadriceps in Humans.**

IRB Number: 5246

Leader of Research Team: **Christopher D. Black**

Address: **1401 Asp Ave., #110 HHC, Norman, OK 73019**

Phone Number**: 405-325-7668 (office); 706-255-3750 (cell)**

If you decide to sign this document, University of Oklahoma (OU) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, and government-issued identification numbers.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to determine if it is safe for you to participate in the exercise used in this study.

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services (HHS), and when required by law. The researchers may also share your PHI with your physician and/or a University of

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

Oklahoma physician in the event of a serious health risk or adverse event that occurs during the study.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OU researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OU.

Canceling Permission. If you give the OU researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OU researchers to use or share your PHI for their research will never end.

Contacting OU: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official	or	Privacy Board
University of Oklahoma PO Box 26901 4300A Oklahoma City, OK 73190		University of Oklahoma 201 Stephenson Pkwy, Suite Norman, OK 73019
If you have questions, call: (405) 271-2511	or	(405) 325-8110

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OU and OU's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

Participant Name (Print): _____

_____ _____

Signature of Participant Date
or Parent if Participant is a minor

Or

_____ _____

Signature of Legal Representative** Date

**If signed by a Legal Representative of the Participant, provide a description of the relationship to the Participant and the authority to act as Legal Representative:

_____

OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

APPENDIX D: HEALTH STATUS QUESTIONNAIRE

Health Status Questionnaire

Part 1. Information about the individual

1. _____

   Participant ID

2. _____

   Date

3. _____ _____

   Mailing Address Phone #

   _____ Email

4. _____ _____

   Primary Physician Physician Phone#

   _____

   Date of Last Physical Examination

5. _____ _____

   Person to contact in emergency Phone

6. Gender (circle one) Female Male

7. Age _____ Date of Birth _____/_____/_____

8. Height _____ Weight_____

9. Do you smoke? Yes No

10. If you are a smoker, indicate number smoked per day:

Cigarettes: 40 or more 20-39 10-19 1-9

Cigars or pipes only: 5 or more or any inhaled Less than 5, none inhaled

11. Are you currently taking prescription or over-the-counter medication(s)? If so, please list the medication, daily dose, and why you are taking it.

12. Are you currently taking any vitamins or nutritional supplements? If so, please list the vitamin/supplement, the daily dose, and why you are taking it.

Part 2. Medical History

You have had or currently have any of the following:

History

___ A heart attack

___ Heart surgery

___ Cardiac catheterization

___ Coronary angioplasty (PTCA)

___ Pacemaker-implantable cardiac defibrillatory/ rhythm disturbance

___ Heart valve disease

___ Heart failure

___ Heart transplantation

___ Congenital heart disease

___ Peripheral arterial disease

___ Stoke

Signs/Symptoms

___ You experience discomfort and/or pain with exertion in the chest, neck, jaw, arms

___ You experience unreasonable breathlessness at rest or with mild exertion

___ You experience dizziness, fainting, or blackouts

___ You experience ankle edema

___ You experience heart palpitations or tachycardia (unpleasant awareness of force or rapid heart beats)

___ You have or experience intermittent claudication (muscle pain due to ischemia)

___ You have a heart murmur

___ You take medication(s) for ANY type of heart condition or high blood pressure

Other health issues

___ You have diabetes

___ You have a thyroid disorder

___ You have a renal (kidney) disorder

___ You have liver disease (e.g. cirrhosis)

___ You have COPD, asthma, cystic fibrosis or other lung disease

___ You have burning or cramping sensation in your lower legs when walking short distances

___ You have musculoskeletal problems that limit your physical activity (arthritis, etc.)

___ You are pregnant

Part III: Cardiovascular Risk Factors

Age

___ You are a man older than 45 years

___ You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal

Medical/Lifestyle

___ You smoke, or quit smoking within the previous 6 months

___ A physician has ever said have high blood pressure (>140/90)?

___ A physician has said you have high cholesterol (Total >200 mg/dl or LDL cholesterol is >130 mg/dl)

___ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)

___ You are physically inactive (i.e., you get <30 minutes of physical activity 3 days per week)

___ You have impaired fasting glucose (> 100mg/dl) that has been confirmed by a doctor on two separate occasions

___ Your BMI is >30 **BMI_____**

I understand my signature signifies that I have read and understand all the information on the questionnaire, that I have truthfully answered all the questions, and that any questions/concerns I may have had have been addressed to my complete satisfaction.

Name (please print)_____

Signature _____Date _____

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(October 2002)

http://www.ipaq.ki.se/ipaq.htm

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

____ **hours per day**

____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

____ **hours per day**

____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

____ **hours per day**

____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

____ **hours per day**

____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

____ **hours per day**

____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days *walking* ***from place to place?***

____ **hours per day**

____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

____ **hours per day**

____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

____ **hours per day**

____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

____ **hours per day**

____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

____ **hours per day**

____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

____ **hours per day**

____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

____ **hours per day**

____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

APPENDIX F: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before starting to become much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by your doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7. Do you know of any other reason why you should not do physical activity?

If you answered

YES to one or more questions

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may able to any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful to you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

▪ Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.	**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT _____ WITNESS _____

Or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

APPENDIX G: TALENT RELEASE

The University of Oklahoma®

TALENT RELEASE

PERFORMER (Name):_____

ADDRESS (Campus or Permanent):_____

CLIENT (Department):_____

JOB NAME: *Photography for departmental publications including but not limited to the department's Web site, promotional brochures, newsletters, postcards, etc.*

For the consideration received, including but not limited to publicity, the adequacy of which is hereby acknowledged, I hereby grant to the Board of Regents of the University of Oklahoma, its successors and assigns, and those acting under the permission, or upon their authority, or those by whom they are commissioned:

(1) The unqualified right and permission to reproduce, copyright, publish, circulate and otherwise use photographs and/or motion pictures of me, and voice reproduction, whether taken in a studio or elsewhere, in black-and-white or in colors, alone or in conjunction with other persons or characters, real or imaginary, in any part of the world including posting on social media sites such as YouTube. I hereby waive the opportunity or right to inspect or approve the finished photographs, films, or tapes or the use to which they may be put or the copy or illustrations used in connection therewith. This release covers composite, stunt, comic, freak, or any unusual photograph and/or motion picture or voice reproduction caused by optical illusion, distortion or alteration or made by retouching or by using parts of several photographs or by any other method. All such use shall be for the purpose of promoting, supporting, or otherwise furthering the mission of the University.

(2) All my right, title and interest in and to all negatives, prints, tapes, and reproductions thereof, and I so hereby release the aforesaid parties and their successors and assigns, if any, from any and all rights, claims, demands, actions, or suits which I may or can have against them on account of the use of publication of said photographs and/or motion pictures or tapes. I have read and understood the release stated above and do hereby agree to its terms and conditions.

SIGNATURE:_____

STUDENT ID NO.:_____ DATE:_____

APPENDIX H: PHOTO RELEASE

THE UNIVERSITY OF OKLAHOMA PHOTO RELEASE

Please check one of the following to indicate proper copyright authorization for photo reprint use in publications or for publicity by the University of Oklahoma:

❑ Attached is copyright permission from the photographer to use

_____

_____

photo for the following purpose(s): _____

_____

_____

❑ I own copyright to _____

_____

photo and give permission for the following use(s): _____

_____

_____

❑ I have permission from _____

(photographer magazine, etc.) to use

_____

_____

photo for the following purpose(s):

_____

_____

Signature Date